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## DAIRY RESEARCH PROGRESS REPORT

### Part I-b of

### Animal-Poultry and Products Research

A summary of current program and preliminary report of progress of the United States Department of Agriculture and related work of the State Agricultural Experiment Stations.

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE  
Washington, D. C. 20250

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# Part I-b, DAIRY RESEARCH PROGRESS REPORT

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The research program pertaining to dairy is reported in two volumes: Part I-b and Part II of Animal-Poultry and Products Research. This volume contains a report of farm research that applies primarily to dairy production; however, some of the research, particularly diseases and parasites, could apply to beef cattle as well. The companion volume, Part II, contains information that may be applicable to other classes of livestock.

The following subject matter progress reports are prepared by U.S.D.A. The number prefixes refer to advisory committees listed later that review the research reported:

- 6 - Forestry (other than Forest Service)
- 7 - Beef Cattle, Part I-a
- 7 - Dairy, Part I-b
- 7 - Poultry, Part I-c
- 7 - Sheep and Wool, Part I-d
- 7 - Swine, Part I-e
- 7 - Animal-Poultry and Products, Part II
- 8 - Cotton and Cottonseed
- 9 - Grain and Forage Crops
- 10 - Horticultural Crops
- 11 - Oilseed and Peanut
- 11 - Sugar
- 13 - Tobacco

The information contained in the above subject matter reports was first reported in the following organizational unit reports. As above, the number prefixes refer to advisory committees listed later that review all of the work of the respective divisions or services.

#### Agricultural Research Service (ARS)

- 1 - Agricultural Engineering
- 1 - Soil and Water Conservation
- 2 - Utilization -- Eastern
- 2 - Utilization -- Northern
- 2 - Utilization -- Southern
- 2 - Utilization -- Western
- 3 - Human Nutrition
- 3 - Clothing and Housing
- 3 - Consumer and Food Economics
- 4 - Market Quality
- 4 - Transportation and Facilities
- 7 - Animal Husbandry
- 7 - Animal Disease and Parasite
- 12 - Crops
- 12 - Entomology

#### Economic Research Service (ERS)

- 1, 5 - Economic Development
- 4, 5 - Marketing Economics
- 5 - Farm Production Economics
- 5 - Economic and Statistical Analysis
- 5 - Foreign Development and Trade
- 5 - Foreign Regional Analysis
- 5 - Natural Resource Economics
- 6 - Forest Service - Research (FS)
- 4, 5 - Farmer Cooperative Service (FCS)
- 4, 5 - Statistical Reporting Service (SRS)



The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

1. Farm Resources and Facilities Research
2. Utilization Research and Development
3. Human Nutrition and Consumer Use Research
4. Marketing Research
5. Agricultural Economics Research
6. Forestry Research
7. Animal and Animal Products Research
8. Cotton Research
9. Grain and Forage Crops Research
10. Horticultural Crops Research
11. Oilseed, Peanut and Sugar Crops Research
12. Plant Science and Entomology Research
13. Tobacco Research

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C. 20250

## INTRODUCTION

The dairy research program reported in Part I-b and Part II, Animal-Poultry and Products Progress Reports, covers work directly related to the production, processing, distribution, and consumption of milk and its products. The information has been assembled from the organizational unit reports of the several divisions. This report does not include extensive cross commodity work, much of which is basic in character, which contributes to the solution of not only dairy problems but also to the problems of other commodities. Progress on cross commodity work is found in the organization's unit reports of the several divisions.

These reports are organized by "Problem Areas" which are shown in the table of contents. For each area there is a statement of (1) the problem, (2) the USDA program, (3) State experiment station programs, (4) a summary of progress during the past year on USDA, and cooperative work, and (5) a list of publications resulting from USDA and cooperative work.

Research on animal-poultry and products problems is supported by (1) Federal funds appropriated to the research agencies of the USDA, (2) Federal and State funds appropriated to the State agricultural experiment stations, and (3) private funds for research carried on in private laboratories or for support of State station and USDA work.

### Research by USDA

Farm research pertaining to dairy is conducted in the Agricultural Research Service Divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, diseases and parasites, insects, housing and management of dairy animals, and sanitary handling and storing of milk until it leaves the farm. The work involves 169 professional man-years of scientific effort which includes research on cattle diseases and parasites that is applicable to beef cattle, also, and which amounts to 29 man-years on infectious diseases, 20 on parasites, and 24 on foot-and-mouth disease.

Nutrition, consumer, and utilization research pertaining to dairy is conducted in the Agricultural Research Service Divisions of Human Nutrition, Consumer and Food Economics, and Eastern Utilization. The work comprises investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions and with the processing phase involving pasteurization, bottling, separation of cream and skim milk; manufacture into products such as butter, powder, cheese, concentrated forms, ice cream, and numerous specialty items. Also, it is concerned with improved equipment and processes. The work in these divisions applicable to dairy is estimated at 94 professional man-years of scientific effort.



Marketing and economic research pertaining to dairy is carried on within four services: Agricultural Research Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing, and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, ARS; Transportation and Facilities, ARS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The scientific effort involved by these divisions applicable to dairy is estimated at 24 professional man-years.

#### Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects dairy research is carried on by 52 State Experiment Stations. The types of work to which the largest amount of effort is devoted include nutrition and management, diseases, breeding, physiology, utilization and marketing. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.

Privately supported dairy research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of basic work done by State Station and Department scientists.

A number of companies make application of basic research developed through public research on products intended for prevention, control, or treatment of diseases, parasites and insects; however, final evaluation is often done cooperatively with public agencies. Most of the identification and classification of insects, diseases and parasites is done by public institutions but the information is available for use by individuals and firms.

About 1/3 of the industry research effort in the dairy industry is in the utilization field. Very little of the work is basic and where it is, the results are usually patented. In applied research the major activities are in cost saving, container testing and low calorie product formulation. Public research was used to develop the procedure for removal of strontium-90 from milk.



The contributions of dairy producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer herds and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situation. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

### **Examples of Recent Research Accomplishments by USDA and Cooperating Scientists**

Method for making genetic progress for milk yield. A method for evaluating DHIA cows and identifying those of outstanding breeding value for extensive use was initiated recently. The procedure considers the cow's deviation from herd mates, her paternal half-sister's deviation from herd mates, and adjusts for the number of milk and milk-fat records of the cow and daughters of her sire. Of the more than 1/2 million cows evaluated annually by AHRD, the top 2% are identified and the results made available to the dairy industry, along with genetic appraisals of sires in DHIA. These estimates of breeding value, when fully and effectively implemented through artificial insemination, can more than double the current rate of genetic progress for milk yield in the Nation's dairy herds.

(4) Ventilation of livestock buildings--Research in cooperation with State Experiment Stations has obtained much needed basic data on the heat and moisture given off by cattle, hogs, and poultry, and on the influence of building environment on production and feed consumption. The heat and moisture dissipation data are considered basic design data for ventilation systems of poultry, dairy, and swine buildings. They appear in design handbooks including the 1965 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating, and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

Genetic Variants of Cow's Milk Caseins Analyzed. Casein, the most abundant protein in milk, is made up of many related but chemically distinct kinds of molecules. Some caseins show heritability characteristics which prove them to be genetic variants of one another. These genetically variant molecules are being analyzed for the numbers of the several amino acids, which are the compositional units that make up the large chainlike molecule of the protein.

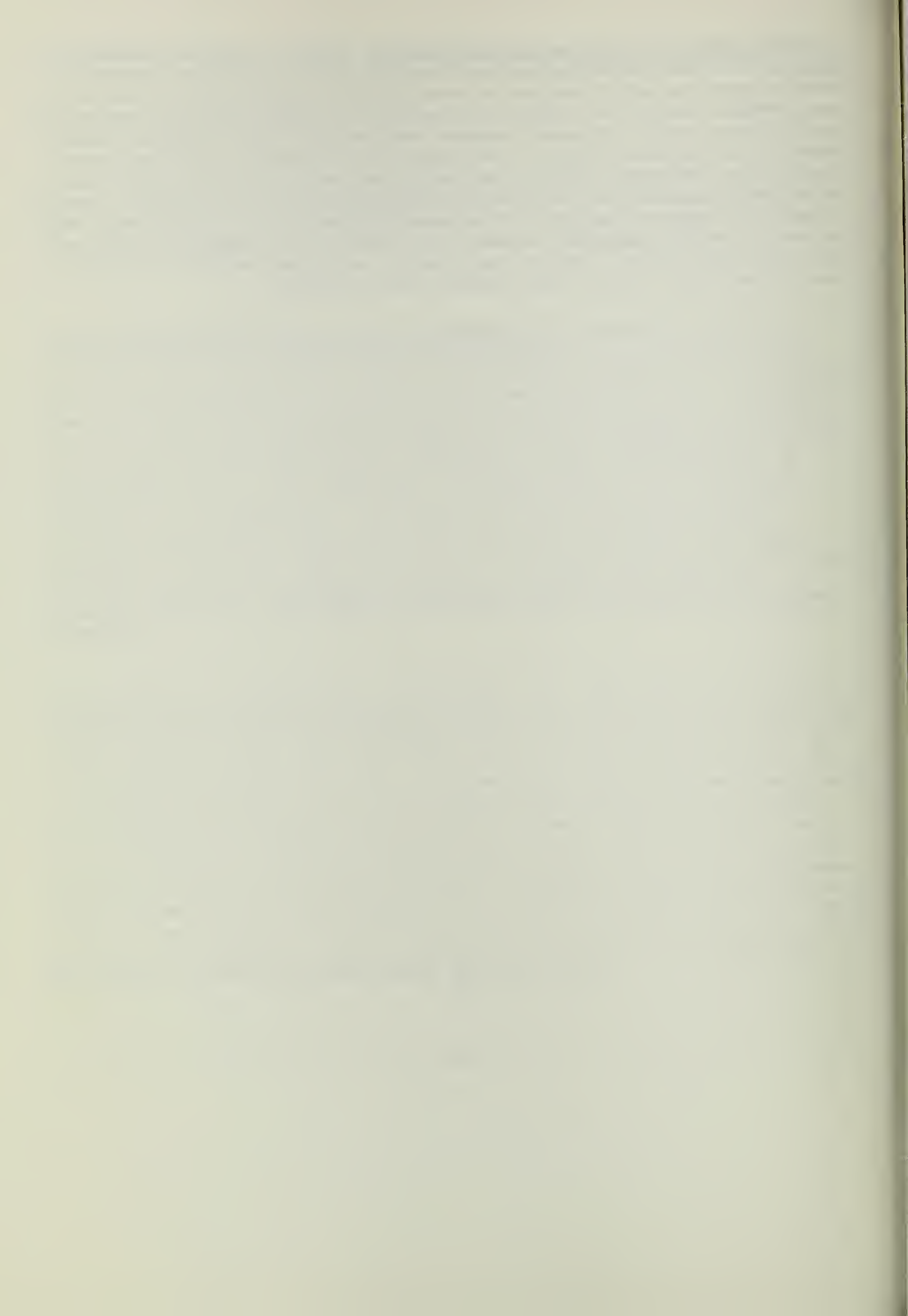


Multiple substitution of amino acid residues is indicated for some of the variants. Compositional differences among the genetic variants are being related to their facility in forming the molecular aggregates or micelles which contribute the normal whiteness and opacity to milk, and which sometimes aggregate further to form undesirable gels or insoluble sediment. The basic information being acquired in this study may have practical value in accounting for geographic, breed and herd differences in the processing properties of milk. It will be useful in developing means for controlling the aggregation of proteins in processed milks. This work is being done in collaboration with scientists of the Dairy Cattle Research Branch of ARS.

Strontium 90 Removal Process Tested Commercially. Commercial adaptation of the Beltsville fixed-bed cationic ion-exchange method of removing radiostrontium from milk has been achieved successfully through contract research. The milk was processed at a rate of 12,500 pounds per hour in an established commercial dairy which had been equipped with strontium removal equipment. More than 90% of the environmental radiostrontium was removed from the milk without appreciably affecting its appearance, flavor, wholesomeness, or nutritional quality. The additional equipment required can be readily integrated into commercial milk bottling operations without significant increase in processing time. The additional cost for removing radiostrontium from milk is calculated to be from one to two cents per quart, depending on the daily volume of milk being processed and the extent of reuse of removal materials. The method provides a feasible and practical **"standby" means for commercially processing milk in the event of a nuclear emergency.**

Dietary Factors in Milk Flavor Investigated. P.L.-480-supported research in Finland is providing new information on the effect of diet on the flavor and quality of cow's milk. Cows maintained on a purified protein-free diet are producing a so-called "zero" milk which serves as a basis for studying the origin of milk flavors. To date, experiments have been concerned mostly with a comparison of milks resulting from protein-free and protein-rich diets. These milks are strikingly similar in their "normal" taste and smell and in their composition. Analysis indicates that even the protein fractions are similar, although slight differences in fat contents and volatile substances do exist and will be investigated further. Extensive studies on the effect of flavor components in certain fodders, in the rumen contents, and in the cow's blood are now underway. Future findings from this research will be of special value to milk processors, who are extremely interested in the origin and **and control of flavor in concentrated and dried milks.**

Bacterial Rennets for Cheesemaking Developed. Indian scientists working under a P.L.-480 research grant have reported promising results toward production of bacterial enzymes as substitutes for calf rennet in cheesemaking where its use is restricted by religious beliefs in large areas of the world. Four hundred microbial rennets were prepared and studied for their possible use in cheesemaking. Five emerged as potentially good rennet producers. An additional highlight of this study was the discovery that the phytic acid contained in wheat bran, a cheap agricultural by-product, greatly stimulates enzyme production. It is expected that, as a result of this and related research, bacterial rennets will eventually be produced industrially and that their use will lead to new domestic and export markets for cheese among groups with special food problems and prejudices.





## I. FARM RESEARCH

### DAIRY CATTLE - BREEDING Animal Husbandry Research Division, ARS

Problem. Dairymen need information on improved genetic methods for increasing and changing productive performance and efficiency in order to reduce unit costs and to meet current and future market demands. Precise information is needed on the relative importance of performance traits, the heritability of certain traits, sources of variation, nature of inheritance, response to selection, and systems of mating. Research is needed on milk and blood constituents, specific and general combining ability, heterosis and adaptability in order to reveal new genetic traits of economic importance and to develop new genetic methods of bringing about livestock improvement.

#### USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by geneticists on basic and applied studies of the inheritance of the dairy cow, including experiments designed for evaluating the application of advanced genetic concepts to dairy cattle improvement. The work is in progress at Beltsville, Maryland, and cooperatively with experiment stations in 14 States and in laboratories in 17 foreign countries. Several of the studies contribute to the North Central and Southern regional dairy cattle breeding projects. Cooperation is also carried out with the National Association of Animal Breeders and with the various dairy cattle breed registry organizations.

The Federal scientific effort devoted to the research in this area totals 17.1 professional man-years. Of this number, 6.6 are devoted to genetics and interrelations of performance traits, and 10.5 to selection and systems of breeding.

A contract in the amount of \$20,000 per year is in effect with the Wisconsin Agricultural Experiment Station to study the meat production potential of dairy cattle. Its duration is four years, 1964-67.

A grant with the Agricultural Research Center, Tikkurila, Finland, provides for research on breed differences relating to antigenic properties of cattle blood, their inheritance relating to economic characteristics and genetic origin of the breeds. Its duration is for four years, 1961-65, and involves PL 480 funds with a \$61,804 equivalent in Finnish Finmarks.

A PL 480 grant with the Division of Investigaciones Agropecuarias, Ministry of Agriculture, Turipana, Colombia, for \$246,000 equivalent in Colombian pesos, supports work on the evaluation of the native breed, Costeno Con Cuernos, and Holsteins and Brown Swiss when selected for dairy traits under the hot and humid conditions of Northern Colombia, for the period 1962-67.

## PROGRAM OF STATE EXPERIMENT STATIONS

Dairy cattle breeding research is conducted with State agricultural experiment station and college herds, institutional herds, and privately owned herds; with data available through the DHIA program; and in cooperation with artificial breeding associations in sire proving and similar studies. Areas of investigations include: (1) the estimation of genetic parameters such as heritability, genetic correlations, genetic transmitting ability, and heterosis, and (2) response to programs for genetic improvement. Specific items of study include selection response, milk constituents, crossbreeding, genetic abnormalities, climatic adaptability, blood antigens, milking rate, and efficiency of feed utilization. Much of the research is conducted under two regional projects--NC-2, Improvement of Dairy Cattle Through Breeding, and S-49, Genetic Methods of Improving Dairy Cattle for the South.

The USDA is cooperating on several research projects with the State agricultural experiment stations and participates actively in the two regional projects.

The total research effort on dairy cattle breeding research by the State agricultural experiment stations is 50.0 professional man-years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Genetics and Interrelationships of Performance Traits

1. Relationships between internal and external anatomy and milk production of dairy cattle. Over a period of more than 30 years, cows of various breeds were slaughtered and measured for both external body form and internal anatomy. These studies were made to obtain information that might aid in the identification of potential superiority of performance in cows not having production records. Correlations for all relationships among 77 variables, including external body measurements, internal anatomical weights or measurements, and 6 measures of producing ability were computed on data from 184 Holstein and 195 Jersey cows. The highest correlations between external body measurements and production records included head length, length of withers to hips, length of shoulders to pin bones, height at withers, back and hips, and depth of forechest. No correlations were higher than 0.30. Body widths tended to be negatively associated with production records.

The highest correlations between internal anatomical weights or measurements and production records included those for weights of blood, heart, lungs, stomach, liver, kidneys, and length of intestines. These correlations were generally higher than those for external measurements. Production correlations with weights of thoracic and abdominal fat and carcass weight were distinctly negative. Correlations with weights of endocrine glands were low. (AH gl-2)



2. Relationships between internal and external anatomical characteristics. These studies were made to determine the relationships between certain characteristics in the external conformation of dairy cows, the dimensions of the thoracic cavity, and the relative sizes of various internal organs and glands that are essential to milk production. The highest correlations obtained for weights of heart, lungs, blood, and kidneys were with body length, height, head length, and body depth. The highest correlations involving weights of stomach, liver, spleen, and intestine length were with liveweight, body circumference, and body width. These external measurements had higher correlations with weights of thoracic and abdominal fat and carcass weight than with size of stomach, intestines, liver, or spleen. However, the correlations were nearly all of small magnitude and give little support to theories that one or a few external measurements are uniquely indicative of the size of an internal organ or anatomical structure. The usefulness of external measurements as an indicator of internal organ size is questionable. (AH gl-2)

3. Age effect on anatomical structures. A study of the effect of age at slaughter on external body measurements and internal anatomical weights and measurements was made on data from 350 Holstein and 346 Jersey cows at Beltsville and State experiment stations. Age at slaughter ranged from 4-10 years. Results from analysis of variance were not uniform but provide several general conclusions.

The greatest increases with age among external body measurements were for body depth, wedge in depth, body length, hip width, muzzle circumference, and head length. Body width, forehead width, pinbone width, heights at hips and pinbones, and shinbone circumference were least affected.

The greatest age effect on internal anatomical items were for stomach weight; intestine length; lung, blood, and kidney weights; and weights of pituitary, thyroid, adrenals, and thymus. Age had little effect on brain weight, carcass weight, and weights of thoracic and abdominal fat. (AH gl-2)

4. Genetic-environmental interactions in performance characteristics. In cooperation with the Tennessee Agricultural Experiment Station at Lewisburg, Tennessee, information has been accumulated on the importance of genetic-environmental interactions. A total of 101 Jersey cows on a ration of forage plus grain, and 104 on a ration of forage only have completed first lactations. These cows represent 13 different sire groups. The average M.E. FCM yield of the forage plus grain group was 12,483 pounds as compared to 10,192 pounds for the forage only group. The latter group produced 81.6% as much as the forage plus grain cows. Individual sire groups, however, ranged from 70.5% to 92.6%. The results indicated that sires generally showing superiority in one environment will also show superiority in other environments. There are exceptions however. For example, one sire whose semen was imported from New Zealand ranked 12th on grain plus forage but was 3rd on forage only. He was selected on the



basis of progeny test information in New Zealand which was obtained under conditions of forage only rations.

At Utah a similar experiment is in progress using Holstein cows. Production data for milk, fat, and FCM for 166 daughters of 13 sires have been accumulated. Production (2X-305-day ME) for sire groups on the all forage ration ranged from 7,595 to 10,868 of FCM, 1,056 to 1,403 pounds of total solids, and 291 to 365 pounds of protein. Daughters on the all forage ration produced 69.8%, 70.5%, and 69.3% as much FCM, total solids, and protein, respectively, as their contemporary half-sibs on the normal ration. Values ranged from 57% to 88% for individual sire groups. A statistical analysis showed a highly significant difference between sires and between rations for all measures of production. The sire by ration interaction was significant for the production of protein and total solids, but was non-significant for all other measures of production. The sire contributing most of the interaction was another bull whose semen was imported from New Zealand. Results to date indicate that progress could be made in selecting cattle that will perform better on one ration than another. However, this progress would at best be exceedingly slow.

Reproductive performance was studied with 128 daughters fed forage only or forage plus grain. Eight sire groups were involved. The average number of days from freshening to first heat was 51 for both rations and ranged from 33 to 68 for different sire groups. However, the forage only cows were bred and conceived earlier than the cows on forage plus grain. This occurred despite the fact that services/conception were the same for both rations. (AH gl-4)

5. Ration effects on production efficiency. Analysis of the research at Beltsville to determine the value of certain feeding regimes in estimating genetic differences among cows in feed efficiency is now completed. Mature Holstein cows were divided into two treatment groups. In treatment I, 23 cows were fed at an amount to support 14,000 pounds of FCM yield and 1500 pounds of body weight for the lactation period. In treatment II, 26 cows were fed individually at 110% of Morrison's maximum requirements for production and maintenance.

Although the results indicated that both groups utilized their feed at approximately the same relative efficiency, there was more variation among treatment II cows in all variables except feed efficiency. This was probably because some of the cows in treatment I were overfed and others were underfed relative to their requirements. Further evidence for this is the range in FCM yield in treatment I which was from 9,053 to 17,842 pounds.

The repeatability between successive lactations for FCM yield was 0.577 in treatment I and 0.565 in II. The corresponding values for feed efficiency were 0.354 and 0.404. There was a more similar consecutive lactation response between and within treatment groups in FCM than in feed



efficiency. (AH gl-4)

6. Meat production from beef, dual purpose, and dairy steers. This study was initiated in cooperation with the Beef Cattle Research Branch to determine the relative merits of various breeds of cattle and different management systems in the production of beef. Twenty-eight Holsteins, 23 Milking Shorthorns, 22 Jerseys, and 25 Herefords have completed the second replication of the study. The steers of each breed were divided into three feeding regimes from 180 days to slaughter. The rations were (a) all hay, (b) all hay until 5/6 of slaughter weight was attained and then a fattening ration, and (c) all fattening.

The average daily rate of gain from 180 days to slaughter for all steers fed either the high or low level of nutrition from birth to 180 days was 1.34, 1.51, and 1.65, respectively for the hay, hay-fattening and fattening rations. The Holsteins had the highest average daily rate of gain. They were followed by Milking Shorthorns, Herefords and Jerseys. The same trends were true for efficiency of gain.

The rib eye areas for Holsteins, Jerseys, Milking Shorthorns, and Herefords were 10.4, 9.34, 10.5, and 10.2, respectively. No significant trend due to ration appeared in any of the breeds except Herefords. In this breed, the steers fed a high plane of nutrition from 0-180 days had an average rib eye area of 9.0 as opposed to 11.4 for those fed the low plane, irrespective of the feeding regime after 180 days of age.

Tenderness studies, as evaluated by the Warner Bratzler shear, showed that Jerseys were the most tender, followed by Holsteins, Herefords, and Milking Shorthorns. For all the breeds except Jerseys, the steers fed the high plane of nutrition during the first 6 months and all concentrate from 6 months to slaughter were the most tender. However, Jersey steers fed the low plane of nutrition during the first 6 months and all concentrates from 6 months to slaughter were more tender than any of the steers in the other breeds. (AH d3-6)

7. Genetics of milk constituents. The  $\beta$ -lactoglobulin types of 812 individual cows of various breeds were determined. This work was done in cooperation with the Milk Properties Laboratory, Eastern Utilization Research and Development Division, Philadelphia. The results support the hypothesis that  $\beta$ -lactoglobulins A and B are controlled genetically by codominant autosomal alleles. There appear to be real differences between breeds in the frequencies of the alternative genes. The A and B genes occur in approximately equal numbers in the Holstein and Jersey breeds but all other breeds studied show a preponderance of the B gene.

Milk from five heterozygous cows were analyzed to estimate the relative quantities of  $\beta$ -A and  $\beta$ -B produced by these animals. The results indicate that  $\beta$ -A and  $\beta$ -B are produced in approximately equal amounts, with A being slightly in excess of B.

Lacteal secretions were obtained from two castrated males and typed for  $\beta$ -lactoglobulin. These animals were found to have  $\beta$ -lactoglobulins (B and AB) consistent with their expected genotypes as deduced from pedigree analysis. Lacteal secretions also were obtained from intact male calves at the age of 6 months and from sexually mature bulls. Some of these samples have been typed for genetically controlled casein and  $\beta$ -lactoglobulin variants and the results indicate that normal patterns are obtained. Thus, it appears that it will be feasible to type males directly for these genetic polymorphisms.

Preliminary studies indicate that the kind of  $\alpha_s1$  casein which occurs as a consequence of the mutant gene  $\alpha_s1 - Cn^A$  can cause some physical differences in milk processing. It appears that milk containing A form of  $\alpha_s1$  casein forms a softer curd than milk containing the more common B form. Other differences are also suggested by the preliminary data and are being investigated.

Data on the genetics of the  $\alpha_s1$  and B-casein were pooled with data from workers in Philadelphia, Pennsylvania; Shinfield, England; and Edinburgh, Scotland. Data on 351 Jersey cows indicated that the two systems are not independent and suggested the hypothesis that the two loci are closely linked. The hypothesis was confirmed by family studies carried out independently in France. (AH gl-5)

8. Genetics of blood antigens and other biochemical polymorphisms in dairy cattle. The tenth biennial USDA Comparison Test in cattle blood typing in which 22 laboratories participated was held early in 1965. These laboratories were located in 17 countries. This program, by affording all the laboratories an opportunity to test a portion of each of 40 blood samples, gives an excellent means of comparing blood typing reagents. Determination of serum transferrin and hemoglobin types by electrophoresis was also done. The Beltsville laboratory participated in this latter part of the test and administered the program.

A method for concurrently typing for serum transferrin (Tf) and hemoglobin (Hb) genetic variants was developed at the Beltsville laboratory. A known amount of Hb from an animal is added to a sample of its serum and the mixture is electrophoresed in polyacrylamide gel. With this method, it is possible to determine the types for both of these systems from the stained gel.

Blood typing done by the Ohio State University Cattle Blood Typing Laboratory indicates that the  $\beta$ -lactoglobulin and blood group J loci are linked with 20% or more recombination. Distributions of transmitted gene pairs were as follows: J & A(3), J & B(19), - & A(20), and - & B(7). This distribution is significantly different from the expected distribution. The data failed to indicate that close linkage exists among any of the locus combinations studied. However, since the families studied were small, it is possible that more distant linkage exists but that it was not



detected because of approximately equal numbers of each linkage phase in the group of animals studied.

At Ohio, workers have investigated how each gene in the serum transferrin system provides for the occurrence of three different electrophoretic bands. No immunological difference was found between the different transferrins. Gel filtration and ultra-centrifuge studies of individual transferrins indicate that all of the transferrin forms are approximately the same size. Studies of the intensities of the different transferrin bands in disc gel electrophoresis indicate that the observed polymorphism may be a result of random association of two types of subunits to a genetic base in the molecule. Degree of iron-binding by the transferrin did not appear to be a factor in multiple band formation. (AH g1-6)

Five electrophoretically distinct iron-binding proteins were found in the blood serum of 350 cattle from the Louisiana State University and the Iberia Livestock Experiment Station herds. Three to five components were found in the individual cow sera samples. No significant relationship was found between these transferrin genotypes and breeding efficiency as measured by services per conception in Holstein cows. The transferrin type E was present in Brown Swiss-Jersey crosses but not in purebred Holsteins, Jerseys, Brown Swiss, or Holstein crosses. It was more frequent in the Sindhi crosses than the European crosses. Transferrin types DE and AE were present in the European crossbred cattle and the crosses with Red Sindhi breeding. There were no significant differences in the milk production of animals with different E genotypes in the LSU Holstein herd. Three electrophoretically distinct hemoglobins were found in the sera of cattle. Holsteins had only bovine hemoglobin A. As with transferrin E the frequency of hemoglobin B was greater in crossbred cattle carrying Sindhi breeding than in the European crosses. (AH g4-2)

9. Association with economic traits. The PL 480 project in Finland was completed in April 1965. Considerable difference was found in the frequency of various blood group genes between the Finnish and other Scandinavian breeds, especially in the B blood group system. No association was found between blood groups and the amount of white color in Ayrshire animals. Preliminary analysis of blood type and production information has been made using 81 blood typed Ayrshire bulls and their progeny tests for milk and fat production traits. The studies showed a significant relationship between the B blood group  $O_1A^1$  and fat percent but did not confirm the previously reported association between  $BO_1Y_2D^1$  and  $BO_1Y_1D^1$  and fat percent. The significance of the  $O_1A^1$  association is questioned since a large number of comparisons were made in the analyses and some would be expected to yield statistically significant results on the basis of chance alone. The Finnish workers conclude, on the basis of their preliminary studies, "that the relative importance of blood groups in predicting breeding value for production traits is probably low on the average." Thus, this study is essentially in agreement with others that have been reported. (E8-AH-1)



## B. Selection and Systems of Breeding

1. Comparisons of inbreeding and outbreeding. Accumulation of data on crosses among 6 inbred lines of Holsteins and maintenance of controls has been continued. Results indicated that the increase in production with lactation number was not significantly different between inbreds and non-inbreds. However, most of the inbreds removed from the herd went out because of reproduction failure. This could be an indication that natural selection may eliminate the more homozygous types in older cows through reproductive unfitness. If so, it is possible that under practical conditions differential effects of mating systems regarding increases of production with age would be difficult to detect in any sample. General conclusions from the experiment are that: (a) inbreeding effects on economic traits were not predictable in stocks of different origin, (b) inbred individuals were lower in reproduction and productive merit than outbreds, (c) inbreds within lines were more variable than outbreds in their phenotypic expression of most economic traits, (d) the development and maintenance of inbred lines were costly, and (e) in most cases, the crossing of inbred lines did not produce individuals that were superior to outbred individuals. It appears that intense inbreeding without selection cannot be recommended for application by dairy cattle breeders. (AH g2-5)

2. The influence of parental relationship on the genetic merit of dairy cows and sires. This research was to determine the relative merits of linebreeding, outcrossing, and crossbreeding using progeny tested bulls of high merit as service sires. The actual first lactation FCM averages for the various groups were not significantly different. Results to date indicate that individual sire effects are more important than the effect of breeding systems.

Heifers from each mating system are being fed a standardized ration at the rate of 2.0 pounds per 100 pounds of body weight to determine if there are differences between animals and mating system in feed utilization for growth. The ration consisted of pellets containing 75% alfalfa hay and 25% concentrates. To date, a total of 18 outcrosses, 12 linebreds, and 17 crossbred heifers have finished 120-day feeding trials. The average daily rate of gain was 1.60, 1.50, and 1.62 for these groups, respectively. Corresponding values for efficiency of gain were .156, .152, and 1.54. (AH g2-24)

3. Sire and generation effects on genetic improvement. Data from the Beltsville proven sire project were studied to determine the rate of genetic progress. Each cow was compared to other contemporary cows belonging to the immediate preceding generation. The average increase for generations 1 through 8 as measured by 3X-365-day records was 546 pounds of milk and 20.7 pounds of butterfat. The largest increases occurred during the first four generations when production levels were lowest. The actual sire means showed significant differences among sires; however, when the means were expressed as deviations from herdmates, the



significance disappeared. Only 7 sires are included in the analysis.

Rate of genetic progress seemed to be much slower in generations 5 through 11 as measured by 2X-305-day records and was 21 pounds of milk and 12 pounds of butterfat per generation. Twenty-one sires were included in this phase and the differences between them were significant when studied on a deviation from herdmate basis and on an actual production basis.

The importance of using superior sires is demonstrated by comparing USDA AI Sire Summaries with sires used in the Beltsville herd. Most of the sires used since 1940 were chosen on the basis of their proof in one or a few herds. Since that time, however, 15 of the bulls have AI summaries on a large number of daughters in many herds. Comparisons of the AI summaries with the evaluations of the bulls in the Beltsville herd show that the USDA AI Sire evaluation is a good indicator of transmitting ability for production even within single herds. (AH g2-25)

#### 4. Usefulness of heterosis resulting from interbreed matings.

##### (a) Effect of crossbreeding on growth, pregnancy, and production.

A study on the effects of pregnancy, lactation, production, and breed group on growth was made with data from the Illinois cooperative project. In the Holstein x Guernsey crosses, the Holstein inheritance stimulated growth more than the Guernseys. Breed of dam had a more significant effect on growth rate than breed of sire. Since no significant interaction of breed of sire and breed of dam was evident, the genetic effect on growth from 18-48 months is presumed to be largely a result of simple additive genetic action. Fleshing type measurements, such as heart girth and body weight, were more seriously affected from 24-36 months than skeletal measurements, such as body length, wither height, and chest depth. In the second generation (3/4 crosses) no significant effects were noted in the comparison of purebred versus crossbred cows. These results further support the conclusion that growth during 18-48 months is largely unaffected by heterosis from crossbreeding. (AH g2-23)

(b) Effect of crossbreeding on lactation persistency. First lactation records of 373 cows in the University of Illinois crossbreeding project were analyzed for effects of breed, mean milk yield, and month of calving on the within lactation variation for milk yield. The purebred Holsteins had the highest total within-lactation variance, followed by the four crossbred groups. The purebred Guernseys were the lowest. For each 1/16 increase in Holstein influence, there was an increase of 1.48 units in the total within-lactation variance. The linear regression of mean milk yield on successive 10-day periods changed -0.009 for each 1/16 increase in Holstein influence. The effect of month of freshening on the total within-lactation variance indicated a seasonal trend with freshenings from December through June being associated with high total variance and those from July through November with low total variance. A regression of mean milk yield per period on season was -0.73 for January through June and



-0.54 for July through September. Cows calving April-June showed a tendency for late lactation production to persist at a relatively constant level and hence have had the highest persistency. Cows freshening August-November ranked second and those calving December-March and July were lowest. There were no significant interactions of month of freshening and breed groups for any of the variables studied. (AH g2-23)

(c) The relative size and production of first and later calves. A study of the effects of parity on weight, heart girth, wither height, body length at 2, 3, and 4 years of age, on milk and fat production, and fat test showed that parity of birth of the cow had no significant effect on the productive performance or size of the animal. Although the parity constants were, in general, not significantly different from zero, those for second parity were consistently positive, and those for fourth or later parity were consistently negative. (AH g2-23)

(d) Body growth in purebreds and crossbreds. In the Illinois project, the effects of breed group, system of mating, and specific sires on six body measurements taken from 3-48 months of age were studied. Holsteins, Guernseys, and six intermediate crossbred groups were included. In two-breed crosses the breed of dam had a greater influence on body growth than breed of sire. Means for the two crossbred groups exceeded those of the purebreds at all ages and for all six variables. After 24 months of age, these differences were not statistically significant. The second generation (3/4 crosses) were generally intermediate to the two purebred groups but nearer the Holsteins at early ages. Results in the third generation (5/8 crosses) provided further supporting evidence that breed differences are largely additive genetic differences. There is evidence of a significant amount of heterosis for sires up to 24 months of age but not from 30-48 months. In each generation, the crossbred groups with Guernsey sires usually averaged near the population mean and those with Holstein sires averaged above the mean. (AH g2-23)

(e) The effect of crossing Holsteins and Guernseys on productive performance. Actual lactation yields of milk, fat, FCM, and fat test of 379 first calf heifers in the Illinois project were analyzed. Holsteins, Guernseys, and six groups of intermediate crosses were included. Breed of sire produced a larger effect than breed of dam on all four variables, but the interactions were not statistically significant. In generations 2 (3/4-breds) and 3 (5/8-breds) there were statistically significant linear trends from Guernseys through the crossbreds to Holsteins. Little evidence for non-additive genetic effects on lactation was found in these data. However, there was some indication of specific combining ability involving certain sire and female breed groups. All crossbred groups were above Guernseys but below Holsteins in all measures except for fat test. (AH g2-23)

(f) Performance of two-breed crosses among Ayrshire, Brown Swiss, and Holsteins. Preliminary results from the Beltsville project



indicate that average livability of female calves up to 3 months of age for the crossbred groups was the same as for the parental breeds. Losses of Brown Swiss purebreds and Brown Swiss crosses were significantly higher than for the other three groups up to 3 months, but the differences among breed groups were not significant for the percent born that completed one lactation.

The average daily gain in body weight from birth to 3 months of age was significantly higher for Holsteins (1.42 lb.) than for Ayrshires (1.19 lb.) and Brown Swiss (1.21 lb.). Daily gains for Holsteins during this period were also slightly higher than for the two-breed crosses. From 6 to 12 months of age the average daily gain for Ayrshires was significantly lower than for all other groups. These results indicate differences between the pure breeds in patterns of early growth which are largely eliminated by crossing. In general, Holsteins were significantly superior to Ayrshires, Brown Swiss, and Ayrshire-Swiss crosses but somewhat lower in production than the crossbred groups with 1/2 Holstein breeding. Preliminary results indicate that some crossbred combinations produce a little more efficiently than purebreds in first lactation.

There were no significant breed effects in number of days from calving to first heat, days from calving to first service, number of days from first service to conception, calving interval, days open during lactation, and proportion pregnant at 95 days after parturition, although two-breed crosses showed a slight superiority over purebreds. The proportion removed from the herd as non-breeders was nearly the same in all breed groups. (AH g2-23)

5. Genetic methods for developing adaptability. Of 489 terminal pregnancies in the LSU herd from 1959-64, 46.8% were females and 53.2% were males. In this period, 5.9% of the calves were born dead (stillbirths) with almost twice as many male calves being stillborn as females. Of the females born alive, 85, 82, and 74% remained in the herd up to 3 months of age, 17 months, and to first calving, respectively. Losses from 17 months to first calving were attributed primarily to nonbreeding causes. These findings indicate there is much opportunity for improvement in management and disease control.

Efficiency of reproductive performance of Jerseys, Holsteins, 1/2 Red Sindhi crosses, 1/2 Holstein crosses, 1/2 Brown Swiss crosses, and all crossbreds with Sindhi breeding, in the Iberia Livestock Experiment Station herd was studied. The measures used were days from parturition to first estrus; services per conception; days open in lactation; days from first service to conception; the proportion pregnant at 95, 120, and 145 days after calving with 1, 2, or 3 services; calving interval; and gestation length. All measures tended to rank the breed groups in the same order. Mean calving intervals among breed groups ranged from 381 to 409 days. Although the 1/2 Holstein and 1/2 Swiss crosses showed some superiority



in reproductive efficiency, as measured by calving interval, breed group differences were not significant. It does not appear that incorporation of the Red Sindhi enhanced reproductive efficiency under South Louisiana conditions.

Analyses of data from the State Prison herd at Reidsville, Georgia, showed no significant advantage of the crossbreds over Holsteins in rate of growth, productive performance, or reproductive efficiency, although the crossbreds did exceed the mean of the parents in most traits. The mean milk fat percent for crosses was approximately equal to the weighted mean for the parental breeds, but for the crosses milk, fat, and FCM yields were considerably greater. The milk and fat yield of the Jersey-Brown Swiss crosses surpassed the levels of the purebred Jerseys and Brown Swiss. Crosses with Holstein parentage approached the performance of purebred Holsteins in FCM, but were lower in total milk yield. The crosses required slightly fewer services for conception and the calving interval averaged 11 days less. (AH g4-2)

An analysis of production records on 521 Consteno Con Cuernos (a native breed) at the Turipana Station in Colombia showed wide variation in total milk yield and length of lactation. There were significant effects of age on total yield from first and second lactations, but not beyond. Seasonal effects were important for milk yield at all ages. System of milking was very important in relation to total yield. Cows with two lactations showed a significant increase in yield (+7.6%) from first to second lactation but increases in subsequent lactations were only 1%. The average lactation yields for cows calving November to April were 42.2% higher than for cows calving in the other 6 months. The low yields corresponded to the wet season rather than the dry season, a result contrary to the common view about feed supplies in the tropics. Cows milked with calf at foot averaged 1,542 pounds milk and 203 days in lactation, whereas, cows milked without calf averaged only 706 pounds milk and milked 106 days. It was apparent from these data that if the calf dies shortly after birth, the cow should be removed from the herd. There were also indications that the problem of milk let-down is a very serious one in these cattle. Results thus far do not indicate a high degree of dairy merit in this native breed. Preliminary studies of performance of purebred Holsteins in the same herd indicate that they are capable of much higher production levels than the native cattle. (S5-AH-1)

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DAIRY CATTLE - PHYSIOLOGY  
Animal Husbandry Research Division, ARS

Problem. Fundamental physiological research is required as a basis for improving reproductive and lactational performance of cattle. Breeding failure is a major reason for the disposal of cattle, and results in great economic loss to farmers. Further information is required on the physiological action of hormones in controlling the reproductive process, correcting reproductive abnormalities, and stimulating lactation. Research is also required on physiological processes related to growth and development, nutritional and management requirements, and on heat tolerance of dairy cattle.

USDA AND COOPERATIVE PROGRAM

This is a continuing program, largely in basic research, conducted by physiologists and biochemists. The program is designed to elucidate the reproductive and lactational physiology of cattle, utilizing physiological and biochemical techniques, and to determine physiological mechanisms related to heat tolerance. The work is in progress at Beltsville, Maryland, and cooperatively at the Wisconsin, New York, Massachusetts, Texas, Louisiana, and Georgia Agricultural Experiment Stations. It is coordinated with the NE-41, W-49, and S-49 regional projects.

The Federal scientific effort devoted to the research in this area totals 8.7 professional man-years. Of this number, 3.9 are devoted to the physiology of reproduction, 2.0 to the physiology of milk secretion, 1.0 to the physiology of growth and development, and 1.8 to environmental physiology.

A PL 480 grant at Picassununga, Brazil, provides for research on the anatomical and physiological characteristics affecting heat production and loss of Zebu, European and Zebu-European crossbred cattle and the nature and method of controlling the inheritance. Its duration is five years, 1961-66, with funds of \$63,293 equivalent in Brazilian Cruzeiros. (Also in Area 5)

A project with the Veterinary Institute, Beit Dagan, Israel, in the Department of Reproduction, concerns work on a project entitled, "Comparative Studies of Repeat Breeders and Normal Cows and Heifers." It is for a five-year period and involves PL 480 funds to the extent of \$124,600 equivalent in Israeli pounds.

A PL 480 project with the Department of Applied Pharmacology, The Hebrew University, Rehovoth, Israel, on the mechanism of lactation and its augmentation by hypothalamic stimulation is in progress. It is supported for five years and is for \$126,767 equivalent in Israeli pounds.



A PL 480 project at Izatanagar, India, and one at Karnal, India, are in progress and relate to environmental physiology.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Two regional projects NE-41, "Endocrine Factors Affecting Reproduction in the Bovine Female," and W-49, "Physiological Factors Affecting Fertility in Cattle" are concerned with this program. The work includes many basic studies on hormone methodology and interrelationships directed to determining mechanisms related to fertility so that losses from infertility may be reduced. Improvements in semen preservation continue to be sought.

Work also continues on the physiology of mammary gland growth and milk secretion with considerable emphasis on hormonal and other physiological influences. A portion of this work is also related to rumen function studies and intermediary metabolism of rumen metabolites as these affect both quantity and quality of milk. Physiological factors affecting mastitis are also being studied.

Other physiological work includes studies on milk fever, ketosis and other metabolic diseases, studies on genetic polymorphisms in blood and milk which may be related to production and work on the effects of environmental influences on physiological processes and production of dairy cattle. Animal behavior studies in relation to management and production problems of cattle are also in progress.

The research effort of the States on dairy cattle physiology totals 48.0 professional man-years. An additional 32.0 professional man-years are pertinent to Area 1 (Animal Biology) and are reported therein.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Physiology of Reproduction

1. Intrauterine inoculation with E. coli. The effects of repeated intrauterine inoculation with viable E. coli organisms upon the life span of induced corpora lutea and upon the uteri of 20 postparturient cows were studied. The possible modifying action of the suckling stimulus on these effects was also studied. Bacterial inoculation and the absence of suckling interacted to prolong the life of the corpus luteum. Animals in this treatment group did not return to estrus during a 27-day period. Inoculation increased corpus luteum weight and progesterone content.

E. coli inoculation increased the mean uterine weight at 41 days postpartum; the relative numbers of lymphocytes, plasma cells and polymorphonuclear leucocytes in the endometrium; the dilation and degeneration

of endometrial glands; and the amount of epithelial loss from the endometrium. Suckling decreased the mean uterine weight at 41 days postpartum, the degree of capillary congestion and gland degeneration in the endometrium, and the number of plasma cells present. Lymphocyte numbers were increased by suckling and by E. coli inoculation. (AH h5-6)

## 2. Progesterone administration during early part of estrous cycle.

The effect of exogenous progesterone on the estrous cycle length was studied in the cow, ewe, and gilt. Daily subcutaneous injections of progesterone in corn oil were begun on the first day of estrus. The number of animals, dose per day, and number of days injected were: 12 cows, 100 mg., 10 days; 12 ewes, 25 mg., 6 days; and 14 gilts, 200 mg., 10 days. The average control and treated cycle lengths were: cow, 20.7 and 16.7 days; ewe, 16.5 and 12.7 days; and gilt, 20.7 and 22.8 days. The treatment shortened the estrous cycle in the cow and ewe, but had no significant effect on cycle length in the gilt. (AH h5-6)

## B. Physiology of Milk Secretion

1. Glycogen in normal and mastitic milk. The marked increase observed in the glycogen concentration of mastitic milk, as measured by the anthrone reaction, led to a detailed consideration of whether this anthrone positive material was actually glycogen or some other material which reacts non-specifically in the chemical determination. The behavior of several milk proteins, nucleic acids, and carbohydrates was studied when carried through the same milk glycogen procedure. Casein and lactalbumin gave a slight reaction with the anthrone reagent but  $\beta$ -lactoglobulin, bovine serum albumen, DNA, RNA, and lactose were negative. Use of a hydrolysis procedure and an enzymatic determination for glucose demonstrated that the compound present in normal and mastitic milk was actually glycogen. The protein, pH, lactose, chloride, and histamine content of mastitic milk was also studied. Large increases were observed in chloride and histamine, and a fairly large decrease in lactose. (AH g3-8)

2. The mechanism of lactation and its augmentation by hypothalamic stimulation. The interrelationship of the hypothalamus and the anterior pituitary gland in stimulating lactation was studied in Israel by depressing the activity of the hypothalamus with tranquilizers and other drugs. Forty-six phenothiazine derivatives were studied in an attempt to stimulate lactation without undue general depressant effects. Perphenazine sulfoxide was found to be especially effective in promoting lactation without tranquilizing. Stereotactic studies were undertaken to localize the site of a prolactin-inhibiting factor. Stereotoxic introduction of canules with the lactogenic phenothiazine derivatives into the hypothalamus of rabbits produced copious lactation. These experiments



demonstrated that the prolactin-inhibiting factor was located in the posterior basal region of the hypothalamus, and that perphenazine, by eliminating the hypothalamic prolactin-inhibiting factor, can initiate release of prolactin and cause lactation. (A10-AH-3)

3. Lactose content of the mammary gland. Lactose changes were studied at 5-day intervals during pregnancy, parturition, and lactation in primiparous rats. A method was developed for the separation of lactose from mono- and poly-saccharides by charcoal-celite chromatography, and applied to milk and mammary gland samples. Contrary to previous reports in the literature, little or no lactose was detected in the pregnant non-lactating gland. As growth of the mammary gland proceeded there was a steady decline in the lipid of the gland, from 65% for the non-pregnant gland to 30% at parturition. Milk lactose increased from 1.2 to 2.2 g/100 ml during 20 days of lactation. (AH g3-8)

### C. Environmental Physiology

1. Effects of heat stress on the blood proteins. Two experiments were conducted at Louisiana State University to determine the effect of heat stress on total blood serum protein concentration, electrophoretic components of the serum proteins, changes in hematological attributes, and growth responses of Holstein heifers. Eight hours of exposure to a temperature of 95°F. resulted in no significant changes in blood constituents compared to exposure to 65°F. Heifers were exposed to a period of constant 65°F. temperature, followed by a period of cyclic hot conditions (75, 85, and 95°F.), and a third period of constant 65°F. Blood hematocrit values were significantly lower during the hot period than in the two cool periods while total serum proteins were significantly higher. Albumin, alpha-globulin, beta-globulin, and gamma-globulin showed no marked change. Thus, it appears that there is no disturbance or alteration in the biochemical mechanism of synthesis or catabolism of blood serum protein fractions under heat stress. Daily body weight gains were lowered during the cyclic hot period. (AH g4-1)

2. Rates of moisture evaporation from the surface of cattle. At Texas A&M University sweating rates were obtained on 3 age groups by measuring moisture loss and salt accumulation on the skin. Estimates average evaporation rates for Holsteins were significantly higher than for Jerseys, and Jersey-Brahma crosses. Rates for first lactation animals were somewhat higher than for yearling heifers and mature cows. As the average daily ambient temperature rose from 73-83°F., evaporation rates for Holsteins, Jerseys, and the crosses increased 6.8, 1.2, and 1.0%, respectively. Average skin temperatures for the three breed groups were 100.6, 99.6, and 99.0°F. Skin temperatures were more closely associated ( $r = +0.99$ ) with evaporation rates than respiration and pulse rates or rectal temperature. Salts accumulated on the skin of the Holsteins was significantly higher than for

the Jerseys or crosses. The relation of salt accumulation on the skin to sweating rate suggests that changes in salt concentration on the surface may be used in estimating the sweating rates of cattle. (AH g<sup>4</sup>-1)

3. Use of tympanic membrane temperature for determining response to heat stress. Since temperature near the tympanic membrane of the inner ear has proven more accurate than rectal temperature for assessing man's response to heat stress, this method of measurement and its relation to rectal temperature in cattle was explored. Tympanic and rectal temperatures recorded at 2-minute intervals on 10 cows during 6 hours of changing air temperatures were used for comparisons. Tympanic temperature was much less variable and significantly lower at all air temperatures than rectal temperature. An increase in chamber temperature was sensed at the tympanic membrane within 2-4 minutes, whereas a rise in rectal temperature was not evident in most animals for about 20 minutes. Rectal temperature also lagged behind when chamber temperature was lowered. Differences among cows, methods, and periods were important as well as cow-by-period and method-by-period interactions. Fistulated cows, with most of the rumen contents removed, were exposed to heat stress until 1°C. rise in body temperature was obtained. While room conditions were continued at 42°C., five kg. of ice were placed in the rumen. Again tympanic temperature responded by a marked decline within 2-4 minutes but a decline in rectal temperature was not evident for approximately 20 minutes. Hypothermic studies indicate that speed of response is more important than absolute temperature in identifying the mechanisms which are brought into play as counter measures. These studies show that temperature changes brought about by either external or internal stimuli can be identified more rapidly near the tympanic membrane than by rectal measurements. (AH g<sup>4</sup>-1)

4. Effect of dietary fats and heat stress on the fatty acid composition of milk. Twelve Holstein cows were used to test the effect on 9 milk fat acids when 10% saturated or unsaturated (crude soybean oil) fat is added to the concentrate ration under cool (50-60°F.) and high (90°F.) temperatures. Under cool conditions the high fat rations depressed the proportions of C<sub>12</sub> through C<sub>16</sub> milk fat acids. Palmeric and palmitoleic acid levels were depressed most by the unsaturated oil. Stearic, oleic, and linoleic acids were increased by both high fat rations. The unsaturated oil was most influential. The 90° temperature depressed the levels of lauric, myristic, tetradecenoic, pentadecenoic, palmitoleic, and linoleic acids but increased the levels of saturated palmitic and stearic acids. In these tests ration effects were more important than temperature effects on the proportion of milk fat acids, but the reverse was true for total milk fat secretion. Among-cow variance was not significant for changes in milk fat acids, indicating that all cows responded similarly. (AH g<sup>4</sup>-1)

5. The effect of thymectomy on growth and antibody production of calves. Six Holstein male calves were thymectomized 24-48 hours after birth and 6 pairmates had sham operations for studies of the role of the thymus gland in early immunological defense mechanism development. There were no discernible differences between pairmates in blood cell



counts and hematocrits, made at weekly intervals. At 3 months of age, all calves were challenged with whole sheep blood at weekly intervals for 4 weeks. The antibody titers for the thymectomized calves were approximately 64% of the level in the pairmates the first week following the initial injection of sheep cells. However, after the fourth challenge there was little difference in the antibody titers of the two groups. This indicates that thymectomy impaired but did not destroy antibody production. The thymectomized group also had a greater incidence of scours in the first 3 months and lower rates of gain from 30-120 days. Thus, it appears that the thymus gland is important in postnatal development. A technique for removal of both the thoracic and cervical portions of the thymus gland was developed as a part of the study. The more difficult thoracic portion was removed (97% of the total thymus) through the second intercostal space while the calf was lying on its right side. (AH g4-1)

6. Clipping the hair coat to improve heat tolerance. Heifers with the hair coat clipped to less than 1/4 inch were compared to normal coated pairmates under summer conditions and under very high temperatures in a psychrometric laboratory at Pirassununga, Brazil. Clipping did not have a significant effect on body temperature, respiration rate, pulse rate, number of red blood cells, hemaglobin, or hematocrit. Also, clipping did not change the ranking of the breed groups in their response to heat stress. The thickness of the initial coat covering on the animals was 1/2 inch or less with only a small amount of hair being removed. These tests, which were made under humidity conditions of less than 60%, indicate that clipping "short-coated" animals does not improve their comfort under low humidity conditions. (S3-AH-7)

7. Studies of heat tolerance of Indian cattle and buffaloes. Heifers representing 5 breeds of native cattle (Hariana, Sahiwal, Tharparkar, Gir, and Kankrej) have been subjected to various regimes of controlled temperature and humidity at the Indian Veterinary Research Institute, Izatanagar, India. The measures used to evaluate the responses were body and skin temperatures, respiratory rate and volume, pulse rate, and rate of sweat secretion. Within breed group variance was found more important than among group variance for all measurements under cool, hot-dry, or hot-humid conditions. These findings are contrary to the general opinion that the breed differences are obvious. (A7-AH-1)

8. The use of high forage rations for growing calves and lactating Indian-type cows. Studies with Tharparkar, Sahiwal, and Red Sindhi heifers between 7 and 12 months of age showed that during this age span 50% of the concentrate mixture could be replaced with forages without impairing growth. This type of feeding reduced the cost of rearing the calves nearly 50%. The hemaglobin, calcium, and phosphorous values remained normal on the high roughage diets. There were no differences among breeds in response to the feeding systems nor was there a significant interaction between breed, season, and systems of breeding. Preliminary observations with lactating cows also indicate that Indian breeds can

maintain expected milk yields when the protein requirement is supplied solely by home-grown fodder. (A7-AH-6)

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DAIRY CATTLE - NUTRITION AND MANAGEMENT  
Animal Husbandry Research Division, ARS

Problem. Information on the nutritional processes and requirements of dairy cattle is needed to obtain a more precise evaluation and a better understanding of feeds and rations as a basis for improving feeding practices on farms. Shifts in sources of nutrients fed to dairy cattle require studies on the optimum combination and specific supplements needed in order to provide for the most profitable income over feed cost. Dairy men need to reduce the total cost of feed and labor. They need to improve husbandry and farm management practices through more efficient use of equipment, bedding, feeding and milking systems.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by biochemists, nutritionists, and dairy husbandmen and is aimed at increasing basic and applied knowledge in the feeding and managing of dairy cattle. Considerable emphasis is placed on basic research. Specific studies at Beltsville, Maryland, concern the relationship between net energy, metabolizable energy and total digestible nutrient values; the relationship between digestible and chemical composition and solubility of feed constituents; calorimetric techniques; composition of forages at various stages of harvesting and storage; and factors influencing the chemical quality, palatability and feeding value of silages.

Cooperative projects at State experiment stations concern variations in efficiency of forage utilization by dairy heifers at Utah, effectiveness of various ensiling procedures at Tennessee, and influence of management and environmental factors on adaptability of cattle to the Southeast at Georgia (cooperative with Agricultural Engineering).

Scientists at Beltsville are engaged in studies on the environmental conditions and the mechanisms of infection involved in bovine mastitis. In cooperation with Agricultural Engineering, Entomology, and Eastern Utilization, research is in progress on electrically-controlled and operated equipment for reduction of labor in dairy cattle management; on the evaluation and development of physical methods for the control of flies and other dairy cattle pests; and on the relationship between management practices and milk quality, including flavors.

A grant with the Government Agricultural College and Research Institute, Ludhiana, which is affiliated with Punjab University, Chandigarh, Punjab, India, provides for research on factors affecting the utilization of low-grade roughages and production of volatile fatty acids in the rumen of cattle. Its duration is for five years, 1962-67, and involves PL 480 funds with a grant of \$86,598 equivalent in rupees. (Pertains to Area 1 also)



A contract in the amount of \$10,000 per year to evaluate the feeding value of newer corn hybrids is in effect at the Maryland Agricultural Experiment Station. Duration four years.

A contract in the amount of \$25,000 per year to study the effects of level of concentrate feeding on cost of milk production is in effect at Cornell University. Duration four years.

The Federal scientific effort devoted to research in this area totals 28.3 professional man-years. Of these, 8.5 are in digestion and metabolism, 7.4 in forage evaluation and utilization, 3.1 in nutritional requirements, 3.6 in calf feeding, and 5.7 in management practices, equipment, and facilities.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Calves. At what age and why do calves stop absorbing antibodies from the colostrum is still a moot question. Efforts are being made to reduce the milk feeding period. Different levels and kinds of vegetable oils along with emulsifiers, are being tried, especially for veal production. The newer antibiotics as well as digesters (enzyme preparations) are being studied. Others are interested in the synthesis of the B-vitamins in the intestine, in dextran as a source of iron, and still others in the zinc and manganese requirements of young calves.

Pregnancy and lactation. Much more emphasis is now being placed on the condition of the cow at time of freshening including the permeability of the placental membrane to nutrients circulating in the maternal blood stream. Almost every station is still testing the effects of high quality forage on milk production, or in vitro with an artificial rumen. The validity of the Morrison feeding standards are being questioned for high-producing cows. As a result many experimental animals are being challenged with liberal grain feeding immediately on freshening. The effects on the composition of the milk of high grain feeding, including processing of the grain, are also under investigation. Efforts have also been made to increase the fat in the ration, especially unsaturated fats, in the hope of not only producing milk more economically but with more polyunsaturated fats present. High fat rations might also be less heating.

Copper compounds have been injected intravenously to see if they influence the susceptibility of cow's milk to becoming oxidized. Fluorine has been fed at various levels over long periods of time. There is also the problem of nitrogen fertilization on the availability of magnesium in forage, and of nitrates in the plant on the vitamin A reserves after feeding, including their effect on reproduction. What is the volatile compound in ladino clover that sometimes imparts an undesirable flavor to milk? Can these suspected compounds be tagged with a radioactive compound and thereby be traced and identified more readily? Will drugs like tapazole, thyroxine, diiodosalicylic acid, nitrofurazone and many others

benefit the lactating cow?

The total research effort on dairy nutrition and management at State stations is 136.0 professional man-years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### A. Digestion and Metabolism

1. Summarization of all available literature data on energy balance trials with lactating cows. The data from all laboratories throughout the world having conducted energy balance trials with lactating dairy cows (1905-65) were summarized, using a computer program developed for this purpose. This was necessary so as to obtain a common basis for comparing these experimental results. In the past different factors were used by many laboratories, and various "corrections" made interpretation difficult. Units used to express results and reports published in foreign languages also made direct comparisons difficult. These reports showed that almost no studies had been conducted with high-producing cows. They also illustrated that very few experiments had been performed with cows using body fat as the source of energy, a common occurrence during early lactation with high-producing dairy cows. Regression analyses showed that the overall efficiency of utilization of metabolizable energy by lactating cows was 57-67%, depending on whether ME consumed/kg<sup>3/4</sup> or energy balance /kg<sup>3/4</sup> was employed as the independent variable. The maintenance requirements estimated in this manner would be 121.6 kcal ME/kg<sup>3/4</sup>/24 hr. or 56.4 kcal net energy/kg<sup>3/4</sup>/24 hr. (AH h2-8)

2. Results of energy balance trials with lactating cows. At Beltsville, the results of the first series of energy balance trials with lactating dairy cows have been analyzed. The original overall conclusion based on the first 4 trials was confirmed; namely, that ration composition did significantly affect the efficiency of utilization of dietary energy. The absolute values were dependent upon the assumptions used for estimating maintenance requirements and corrections for tissue losses and gains.

Data were analyzed involving rations A (64% alfalfa and 36% concentrates), B (84% alfalfa and 16% concentrate mixture of corn-soybean meal) and C (100% alfalfa). Ration A exceeded B, and B exceeded C in regard to (a) efficiency of utilization of metabolizable energy (ME) for milk production, (b) digestible energy (DE), (c) digestible dry matter, (d) digestible nitrogen, (e) digestible carbon, (f) digestible organic matter, (g) TDN on a dry matter basis, (h) metabolizable energy content, and (i) total rumen concentration of volatile fatty acids. Opposite trends for rations were found for (a) maintenance requirements, (b) metabolizable energy consumed, and (c) rumen pH. Estimated net energy intake and total energy balance values (including milk production plus tissue deposition) were lowest for C but of similar magnitude in A and B. Ration effects were significant for efficiency of utilization of metabolizable energy for



milk yield, maintenance requirements, concentration of propionic n-butyric, iso-valeric and n-valeric acids, and percent of nitrogen intake lost in feces and urine. Non-significant differences due to rations were found for blood ketones, percent milk fat, solids-not-fat, milk protein, total milk produced, body temperature, and percent nitrogen stored in milk and tissue.

These results demonstrate the importance of differences between rations as they affect the efficiency with which TDN, ME, and/or DE are utilized by cows. Factors derived from these data for rations A, B, and C were 2133, 2118, and 2129 for DE/pound TDN and 1746, 1743, and 1753 for ME/pound TDN, respectively. The ME/DE ratio was found to be dependent upon the amount of feed consumed; thus, the commonly used average factor (ME=82% of DE) can be incorrect when applied to rations that are being fed above 2 times maintenance. Derived factors for 5 times maintenance (approximately full feed for a lactating dairy cow) were 86.6, 87.9 and 87.1 for rations A, B and C, respectively.

Results to date show that the methods and equations being developed will enable determining net energy values of feeds and rations directly from current tables of feed composition and with greater accuracy than previously possible. They also indicate that TDN or other average factors now in use are in error and underestimate the feed actually utilized by the animal.

It has been noted that as animals consume greater quantities of feed, the digestibility of the ration decreases. This was noted in these studies, but the degree of depression was not nearly as marked as that found by other workers. It was also found that the depression in digestibility of the rations used in these studies was compensated for by decreased losses in the urine and methane. (AH h2-8)

### 3. Development of chemical methods for determining the nutritive value of feeds and forage.

(a) Cell-wall constituents. A procedure was developed last year which was satisfactory for forages, but not for corn silage or concentrate feedstuffs because of an interference of starch with the neutral detergent reagent. It has been discovered that starch may be easily removed by boiling the sample with 1% salicylic acid, and then with the neutral detergent reagent. This modification remains to be standardized. Such a method with applicability to all feedstuffs would be of considerable importance to feed manufacturers and to those concerned with livestock feeding, including non-ruminants. (AH h2-6)

(b) Sample preparation. Because of the problem of inducing artifacts by oven drying of forage samples, an alternate procedure has been developed for silages and fresh forage, whereby 2 kg. of the forage are frozen and passed through a meat grinder with a 0.25 inch plate. The

resulting mass is mixed in a large plastic bag and sub-sampled. It has been found that samples as small as 2 g. of wet matter are representative of the parent material. This mode of sample preparation is being used in conjunction with the determination of lignin, acid-detergent fiber, cell-wall constituents, total sugar, crude protein, and dry matter determinations.

(c) Studies on the manner in which lignin affects digestibility. Previously, it had been shown that lignin affected the nutritive availability of the cell wall fraction only, and that soluble components and protein are not influenced by lignification. Further analyses and statistical studies show that within the cell-wall fraction, hemicellulose and cellulose fractions have similar digestibilities and that both are highly correlated with the lignin content of acid-detergent fiber. (AH h2-6)

(d) Development of prediction equations for digestibility. A regression equation was developed on the basis of the concept of an availability index. Two factors were recognized in this equation; the quantity of soluble cell contents, and the degree of lignification. A second prediction equation was developed on the principle of summation, in which the completely digestible cell contents are added to an estimated digestible amount of cell walls. From the sum (equal to true digestibility), an estimate of the endogenous excretion of dry matter is subtracted to give apparent digestibility. While this second prediction equation is more involved, it seems to have greater precision in predicting the nutritive value of some kinds of forages. (AH h2-6)

#### 4. Chemicals in milk.

(a) Dimethoate toxicity. The feeding of corn silage treated with a technical grade of dimethoate showed some evidence of toxicity in an earlier trial at Tifton, Georgia. Therefore, a second experiment was conducted using a pure form of dimethoate applied to the corn crop at the rates of 16 and 32 oz. per acre. It appears that dimethoate persists in the silage since about 40% of that present at the time of ensiling was present after 80 days of storage. The intake of silage by the lactating cows was not affected by the residues in the silage. No dimethoate residues were found in the milk, with the limit of detection at about .002 ppm. Blood cholinesterase inhibition was not detected. It was concluded that treatment of corn with as much as 32 oz. per acre will pose no residue toxicity problems with lactating dairy cows. (AH h2-14)

(b) Preparation of milk fat for Heptachlor analysis. The usual method for the separation of fat from milk for insecticide analysis is to separate out the cream with a cream separator, followed by treatment with sodium sulfate and extraction with hexane. The Stanley and Fauvur method was adapted to separate out the milk fat for the analysis. Analysis of samples of milk using both methods of separation gave similar heptachlor epoxide values. The adapted procedure required about one-fourth the time previously required to prepare the fat for analysis. (AH h2-14)



(c) Endosulfan (Thiodan). In studies conducted at Tifton, Georgia, endosulfan was applied to Bermuda grass at the rate of 4, 8, and 16 oz. per acre. After 7 days, the forage was harvested as silage and 100 pounds of corn were added per ton of chopped grass. After a 78-day storage period, the silage contained 0.41, 0.70, and 2.35 ppm. of endosulfan which was 40, 30, and 44% of that in the original silage. The 3 silages along with control silage were fed to 16 lactating cows for a period of 21 days. Milk produced by cows consuming daily adjusted averages of 15.6, 16.6, 16.4, and 17.2 pounds of silage dry matter for the control, 4, 8, and 16 oz. treatments, respectively, was free from detectable endosulfan. A compound found to be endosulfan sulfate was found in the silage. However, none of this compound was detected in the butterfat. (AH h2-14)

## B. Forage Evaluation and Utilization

1. Dry matter losses in bunker and gas tight silos under adverse conditions. Low-moisture orchardgrass was used to obtain a direct comparison of bunker and Harvestore storage with regard to preservation and feeding value. Poor drying weather caused filling to extend over an 8-day period, hence, rather extreme exposure of bunker stored forage occurred before final sealing. Additional exposure was caused by cattle damaging the top seal. Recovery of dry matter for feed was about 72% in the bunker and 84% in the Harvestore. However, there were no appreciable differences in the feeding value of recovered silage. It was concluded that delays likely encountered when storing low moisture grass are particularly detrimental to preservation efficiency of a bunker. However, the effect on feeding value may be rather small. (AH h3-3)

2. Acceptability of low-moisture silage by cattle. The acceptability of low-moisture alfalfa silage from conventional silos, as measured by voluntary intake, was positively correlated with dry matter content over the range of 30-70%. However, intake of dry matter content above 50% usually was not increased. Fermentation was progressively more restricted as forage dry matter content increased, but dry matter digestibility was not significantly affected. Efficient preservation may be achieved at average dry matter contents up to 63%, with occasional unexplained exceptions.

These experiments indicate little reason to strive for dry matter contents above 50%. However, higher contents, which may be encountered unintentionally under practical conditions, can be accommodated without serious disadvantages. (AH h3-3)

3. Summer feeding of low-moisture orchardgrass silage. In this experiment, 52% dry matter orchardgrass was stored in a 10' x 35' concrete silo. Feeding commenced May 16, the day after filling was complete. Silage removed during the first 2 weeks showed a marked tendency to heat, was of poor chemical quality, contained about 10% spoilage, and was rather unpalatable to dairy cows. Following this initial period, quality and



consumption levels were good and little spoilage was found. It appears that summer feeding of conventionally stored low-moisture silage is quite feasible if a 2-week period between filling and feeding is allowed. (AH h3-3)

4. Effect of Tylosin and zinc bacitracin on silage quality. Previous investigations of the effects of the antibiotics Tylosin and zinc bacitracin on improving silage quality were inconclusive, partly because of good quality control while making the silage. The effects of these materials under more adverse conditions were tested by applying them to high nitrogen orchardgrass stored in 4' x 8' silos. Periodic bored samples over a 68-day period indicated no consistent effect on quality. A final sample, taken at 145 days of age, showed that the pH and ammoniacal nitrogen values for control silage were distinctly higher than for either treatment. Of even greater interest was the marked deterioration in quality of all silages between the 68- and 145-day boring. It is rather common practice to choose an arbitrary age such as 30 or 60 days on which to evaluate silage treatments. The implication being that silage quality is stable after this age. These data show that both absolute and relative quality changed markedly after 68 days of age. These findings raise the question of when if ever does a silage become stable in quality and at what age should evaluations be made. (AH h3-3)

5. Estimation of harvesting losses. Lack of precision in determining harvesting loss is associated with error in estimating the amount of original crop, rather than measurements of the amount actually harvested. In cooperation with Forage and Range Crops Research Division, it was found that dry matter of the standing crop per acre could be estimated with standard errors of about 100 to 200 pounds, which amounted to about 3-6% of the mean yield. Between 19 and 40 small strips per two-acre plot were required to obtain this precision. Estimates of the area harvested were made by ground measurements (Coop. A.E.R.D.) and by aerial photography (coop. Forest Insect Laboratory). Differences of about 4.5% were found between methods of measurement. It was concluded that this orthodox approach to harvesting loss measurements would be useful for distinguishing differences of about 20%, but it would be very laborious.

A second approach eliminated the need for strip samples by substituting the total yield of a direct-cut treatment. The problem of area measurement was reduced to the measurement of the linear distance harvested and making certain that the full-cut width of each machine was utilized. Losses of 1.0 and 2.6% were measured in two replications of field-cured baled hay by this method. These small losses may be associated with elimination of raking by use of a windrower. Slow drying (up to 6 days) had a negligible effect on dry matter loss, when done on portable trays with little chance for mechanical loss. (AH h3-3)

6. Effect of drying on silage intake. Previous data have shown that the dry matter of good quality wilted grass silage was not consumed in



as large an amount as the same crop made into hay. When the wilted silage was dried, intake was not improved. New data, however, show that low quality high moisture orchardgrass silage with a high pH was consumed in greater amounts when dried, or dried and then soaked in water. This suggests that intake of poor quality grass silage is improved by drying and that a study of the portion lost in drying, by volatilization of some of the silage constituents at a high pH, might clarify the cause of poor silage intake. It is also apparent that water, per se, in the silage is not the direct factor involved in intake. (AH h3-3)

7. Inhibition of aerobic spoilage in low-moisture silage. Eleven fungicides which are used for other agricultural purposes were screened by observing their ability to control mold growth in finely chopped hay reconstituted to about 50% moisture. The test was conducted in unsealed one-quart jars. The fungicides were added at 500 ppm. All but one failed to control mold growth over a 12-day period. The one effective material was 3,5-dimethyltetrahydro-1, 3, 5, 2H, thiodiazine-2-thione which is used as a soil fumigant sold under the trade name Mylone. Treatment with as little as 125 ppm. was found to prevent visible mold for 20 days in 50% moisture silage stored in unsealed jars.

A wheat bran mixture containing 25% Mylone was used to treat a low-moisture alfalfa stack. A control stack was also used. The efficiency of the treated stack was markedly improved as indicated by a lower percentage of spoilage and greater percentage of good silage. The amount of dry matter lost (gaseous loss) was surprisingly small in both stacks in view of the extensive spoilage. Chemical analyses of the unspoiled silages showed no differences in composition that could be related to treatment. Limited observations showed that similar amounts of silage dry matter were consumed from treated and control silages.

Although not conclusive, it appears that Mylone could be very effective in reducing certain types of spoilage and that the palatability of silage would not be reduced by this treatment. Possible problems of toxicity and milk residues have not been investigated. (AH h3-3)

8. Biochemical studies relating to silage investigations. The chemical quality of ensiled annual forages was studied in 7 sorghums and one each of sudan and millet. Three or four stages of cutting were used. Forages were ensiled in quart jars and assayed after 90 days for pH and ammoniacal nitrogen. The chemical measures of quality were related to the initial sugar, protein, dry matter, buffering capacity, and osmotic pressure of the forages. Covariance analyses showed the sugar-protein interaction to be important in determining pH and ammoniacal nitrogen. It also showed that chemical quality can be predicted by regressions based on sugar, protein, and dry matter.

In crops where difficulty was encountered in producing a good quality silage, the soft dough stage of seed development appeared to be the best

stage for ensiling. (AH h3-1)

### C. Feeding Heifers

1. Factors affecting the intake of hay-crop silage by dairy heifers. When fed as the sole ration to growing dairy heifers, the dry matter intake of direct-cut hay-crop silage is nearly always lower than the intake of the same forage preserved as hay. The present experiments were conducted to accumulate more data on the composition of direct-cut silage and hay, and their respective digestibility and nitrogen retention when fed to dairy heifers.

The direct-cut silage contained less cell-wall material and thus required less space in the ruminant gastrointestinal tract than the corresponding hay. The lignification of the fiber was similar in both forages. Thus, unless the rate of digestion were reduced for silage, relative to hay, an accumulation of insoluble fiber in the rumen would be unlikely.

The energy digestibility was equal for the silage and hay. While the silage-fed and hay-fed animals digested about the same percentage of nitrogen, the silage-fed animals gained less weight and retained more nitrogen per unit of weight gain. These results indicate that the lowered growth of the silage-fed animals was not due to inadequate nitrogen retention but rather to inadequate energy intake. The inadequate energy intake appears to be associated with palatability or taste preference rather than the accumulation of feed residues in the rumen, which is commonly thought to limit hay intake. (AH h1-1)

2. Particle size fractionation of cell-wall constituents. A method has been developed whereby the neutral detergent fiber portion of forages is separated into various particle size fractions by dry sieve analysis. The method consists of the isolation of cell walls by treatment of the sample with neutral detergent reagent followed by acetone and hexane washings. Sufficient sample was used to yield approximately 10 grams of cell walls. Preliminary studies have been made on particle size with feces, rumen contents, and ground forages. Particle size diminution, resulting from pelleting in addition to that of preliminary grinding, has been demonstrated using this method. The values for modulus of fineness of fecal cell walls were greater for animals fed alfalfa hay than for animals fed alfalfa silage. They were also greater for animals fed alfalfa than for those fed timothy. There was no significant effect due to level of intake. However, based on a limited number of observations, it appears that at high levels of intake (3X maintenance) greater proportions of larger particles would be excreted. (AH h1-1)

3. Effect of nitrate on utilization of corn silage carotene. Twelve Holstein bull calves from 91 to 155 days of age, depleted of their vitamin A reserves, were fed a concentrate mixture low in carotene ad libitum and 454 g. skimmed milk powder per day mixed with water. The calves were fed



nitrate ( $\text{KNO}_3$ ) at levels of 0, 147, and 440 mg./kg. body weight and carotene (from corn silage) at levels of 44, 53, 70, and 97  $\mu\text{g}/\text{kg}$ . body weight. Concentration of blood serum carotene, serum vitamin A, and cerebrospinal fluid (CSF) pressures were determined biweekly during the 123-day experiment. The effect of dietary carotene levels on these three variables was linear and significant. There were no significant differences in these variables due to level of nitrate. There were no significant differences among rations for average daily gain and concentrate intake. It was concluded that the added nitrate had no effect on the utilization of corn silage carotene by Holstein calves. Carotene from corn silage was generally as effective as dehydrated alfalfa leaf meal in preventing increased CSF pressure in calves. (AH h1-5)

#### D. Management Practices, Equipment and Facilities

1. Bovine mastitis - cell counts as estimators. Comparisons were made on 1,214 milk samples between direct counting of body cells (leucocytes) and the California Mastitis Test (CMT), which is an indirect method of estimating leucocytosis. Microscopic investigations showed that 22.3% of the samples that gave a CMT reading of 0 contained leucocytes in excess of 21,000 per ml. Milk samples that scored 1, 2, and 3 on the CMT, misclassified 71.6%, 51.8%, and 4.7%, respectively, when compared to direct microscopic counts. These results indicate that the CMT is reliable only as an estimator of leucocytes when the level of leucocytes is in excess of 1,000,000 per ml.

Four successive milk samples were drawn from 2 groups of cows prior to milking. Cell counts and CMT's were conducted on alternate samples. The mean cell count decreased 50% between the earlier and later samples. There was generally a marked decrease in cell count in successive samplings from a quarter and this change was not clearly reflected in the CMT scores. Even in the absence of any detectable infection by microbial pathogens, marked variations in cell count level frequently occur; both within a normal milking of a quarter, and among foremilk samples taken at intervals between milkings. (AH g3-8)

2. Raw milk quality as influenced by rate of cooling. Investigations were continued on the effect of differing rates of cooling in mechanically refrigerated farm bulk tanks on raw milk quality. Studies using milk of a relatively high bacterial count were begun. A small-scale milk handling regime, designed to simulate can storage and transport under conditions of poor sanitary control was used. During each trial a portion of the raw milk from this regime was added to the regular bulk tank supply at each milking.

Studies to date on such milk, containing a natural mixed bacterial flora of approximately 100,000 per ml. (Standard Plate Count), have given results substantially in accord with those obtained on milk of high initial quality. It appears that, on an every-other-day pickup schedule, the cooling rate

can be decreased to the extent that it requires at least 2 hours for the first milking in the tank to reach 50°F. Such a procedure avoids significant bacterial multiplication during the two-day period. These results indicate no justification for the use of maximum blend temperature as a criterion of acceptable milk cooling. This parameter is a very sensitive measure of rate of tank filling, but a most insensitive measure of cooling rate. The cooling rate could be slowed from about 1 to 4 hours and thereby shift the maximum blend temperature by only 6°F. Under minimum satisfactory cooling conditions, judged microbiologically, the maximum blend temperatures were 59 to 60°F., whereas 50°F. is the commonly accepted upper limit. (AH g3-10)

### 3. Physical methods for fly control.

(a) Effectiveness of electrocutor grids around barns. A method involving electrocutor-grid screens placed in windows plus an indoor electrocutor trap with black light lamps was evaluated for effectiveness in controlling fly populations. Two calf barns where large numbers of house and stable flies were present were used. No substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. However, many flies were killed by the grids. When animals were allowed access through open doors to exercise lots, the fly populations in the test barn and the "check" rapidly equalized. (AH g3-12)

(b) Face fly activity around cattle. Outdoor behavior of face flies was studied, both on herds in the field and on a single animal confined in a cage with a known fly population. Results indicate that only a small proportion, usually less than 10-15%, of the total face fly population actually annoys cattle at any given time. Female flies visit the animals much more frequently than males, but males cause some annoyance. Three- and five-day-old females visit the cow more frequently than 1-day-old females. The number of flies present on cattle is most closely related to the activity of the animal. The greatest annoyance occurs when the cattle are resting quietly. Although flies visit the face most frequently, they rest on other parts of the body in considerable numbers. An evident peak of activity of released colonized flies occurred early in the morning, but that of wild flies appeared more evenly throughout the day. All flies left the cattle at dusk when natural light levels were still quite high.

Analysis of sightings of individual flies (3-day-old females) caged with an animal indicated a median for the population for sightings on the entire animal of 11.3%, on the face 6.0%, and on the body 2.3%. These same results show that the number of visits made by an individual fly and the duration of these visits are determined by the time interval between visits. When the minimum interval between fly-sightings was at least 10 minutes, each fly made 5.5 visits per day to the entire animal, the average visit being 16.1 minutes in length; however, when this minimum



interval between fly-sightings was increased to 60 minutes the number of visits was decreased to 1.6 per fly per day, with an average length of 111.8 minutes per visit. The data varied widely and in some cases the standard deviations for averages exceeded the average.

All these characteristics indicate the advisability of further investigating control measures which affect face flies when they are off the cattle. (AH g3-12)

(c) Activity during sunset and nocturnal resting places. Additional studies of the nocturnal habits of face flies confirmed that they rest on the foliage of trees at night. Although the flies are readily attracted (about 80%) to "black light" ultraviolet in confined spaces, those found resting on foliage at night do not respond in this manner. Electrocutor grid traps with black light lamps placed in trees attracted less than 1% of a released population in 48 hours. (AH g3-12)

(d) Mating activity of female face flies. Laboratory tests of the mating activity of female face flies indicate that sterilized males compete effectively with normal males in mating. Also, females appear to mate only once if they are inseminated during their first mating. Examination of females attempting to remate indicate that only 5-10% had not received any sperm during their first mating. This indicates that use of sterilized males should be effective in preventing reproduction. A laboratory trial using a ratio of 8 sterilized males; 1 untreated male, and 1 female resulted in a 94% reduction in pupae. (AH g3-12)

(e) Monochromatic light studies. Additional tests of the responses of a 3-day-old female face fly to monochromatic light confirmed that black light ultraviolet is highly attractive under conditions of confinement, and that wave lengths in the red and yellow spectral regions are unattractive. (AH g3-12)

4. Environmental influences on production. Feeding, management, and production data were collected from 46 DHIA Holstein herds over a 3-1/2 year period at Wisconsin. A total of 155 variables was included in this study to determine the importance of specific environmental influences on production differences among herds. Sixty variables were found to be significantly correlated with herd production, 15 of which were highly significant. These 15 variables were bedding, cow cleanliness, vacuum (deviation from recommendation), pulsation (deviation from recommendation), amount of help while milking, method of stripping, condition of calves, feeding of calves, herdsman'ship of dairyman, percent TDN in hay, quality score of hay, date of cutting of hay, pounds of TDN fed per 1,000 pounds liveweight per day, percent days herd is in milk, and herd size.

As much as 65% of the variation in herd average milk yield was found to be associated with 10 of these influences. Sixty percent of this variation was associated with herdsman'ship, percent days in milk, TDN level, herd

size, pulsation, vacuum, and hay score.

Many of the environmental factors previously assumed to be important determiners of production were found to be unimportant in this study. Such an example is the average interval from udder massage to milking (mean = 2.8 minutes). While this field study may not have adequately measured each of the 155 variables, it was apparent that cows have the biological capacity to react and adjust favorably to generally superior feeding and management practices. They do not necessarily react favorably to certain specific practices which are not implemented according to accepted recommendations.

The use of the environmental index composed of 7 variables appears to bring about marked reduction in variation between herds and hence appears to have considerable potential for use in increasing the reliability of sire appraisals. However, the environmental index will require further testing before extensive field and research use is justified. (AH g5-1)

5. Social relationships among dairy cows. Two groups of 12 cows each were observed in a feed lot for 5 consecutive days and for a second 5-day period one month later. Three observers recorded agonistic behavior according to the categories of contact(excluding use of the head), bunting, forceful, non-forceful and pushing. From the 3,463 contests observed, dominance values were computed for each of the five categories. A social order was established within groups, combining all observations in all categories. Estimates of repeatability for dominance values were .97 from day to day in the same week, and .95 from day to day in different weeks. The data indicated that a definite social order did exist in an established group of dairy cows. Since this order was stable over a period of time, one day's observations can determine this social order. (AH g5-1)

6. Influence of management practices and environmental factors on adaptability

(a) The value of permanent pastures during the summer months for lactating cows. At Louisiana cows receiving alfalfa hay and concentrate showed only a slight decline in milk production during the summer months, whereas, cows on permanent pasture as the sole source of roughage showed marked declines in milk yield with advancing season. The digestibility of pasture herbage was high during the early part of the growing season (April-May); followed by a significant decline during June, July, and early August; and then a slight increase in late August. There were significant positive correlations between quality score and crude protein, quality score and gross energy, and between crude protein and gross energy. However, low correlations were obtained between milk yield and pasture score and between milk yield and crude protein. The correlations between quality score and mean daily temperature, and quality score and lignin of the plants were negative. (AH g4-3)



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**DAIRY PERFORMANCE AND MANAGEMENT RECORDS**  
**Animal Husbandry Research Division, ARS**

Problem. Effective livestock and poultry improvement cannot be accomplished effectively without adequate records of performance and management. In order to have widespread utility, these records must be produced through carefully coordinated programs yielding uniformity in measurements, standardized analytical procedures, and accuracy and integrity in animal identification and genealogy. Continual revision of uniform data collection, reporting and evaluation techniques in accordance with current research findings requires integration of program operations and research. Only in this manner can there be a continual chain of discovery, field testing, and application with the needed genetic and environmental improvement.

**USDA AND COOPERATIVE PROGRAM**

This is a continuing long-term program of performance testing dairy cattle and poultry, including the evaluation of the genetic merit of dairy cows, sires and herds, chickens for egg or meat production, and turkeys. Also included in the program is the control of egg-transmitted poultry diseases. The work on dairy cattle performance testing is cooperative with 50 States and Puerto Rico and the Records and Breeding Committees of the American Dairy Science Association. Cooperation is also carried out with the National Association of Animal Breeders and the various dairy cattle breed registry organizations. The poultry work is cooperative with Official State Agencies in 47 States and with the supervisors of 25 random sample tests in the United States and Canada.

The Federal scientific effort devoted to the programs in this area totals 7.0 professional man-years. Of this number, 5.0 are devoted to dairy cattle performance testing and 2.0 to poultry performance testing.

**PROGRAM OF STATE EXPERIMENT STATIONS**

The effort of the State stations in this area is quite large. It is difficult, however, to make a manpower estimate which would be distinct from that in breeding sections. As the USDA material indicates, much of the effort in DHIA and in poultry testing is cooperative with the States. At several State stations, DHIA records are processed for dairymen on a reimbursable basis. Data derived is used in estimation of genetic parameters, etc., by resident investigators. Similarly, the random sample poultry tests on broiler and egg production strains conducted at State locations provide information on performance and mortality of commercial stocks. Eggs and meat from these poultry tests are used in detailed studies such as strain comparison of chemical constituents and correlations of these items with production traits.



Animal performance and management records with beef cattle, swine, and sheep are frequently obtained through cooperation with producers, extension service, and industry. In several States, swine testing stations have been established and operated on a self-sufficient basis supported by fees. Research personnel often act in an advisory capacity. In many States, programs of on-the-farm performance and progeny testing of beef cattle have been developed. Again, research and extension people frequently cooperate in this endeavor. At central facilities in some States, bulls belonging to producers are tested for growth and feed efficiency often under the direction of research personnel. Similarly, a limited number of ram testing stations have been developed for indicating growth rate.

No estimate of State station professional man-years is made.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### Dairy Cattle

1. Sire evaluation program. A total of 1,685,797 records of performance reported during the year, along with the 11 million records available in magnetic tape files, were used in producing 18,533 individual genetic appraisals of sires. From these, the dairy industry and cooperators were provided with 59,963 sire records. These genetic appraisals were made on a quarterly basis and included all sires qualifying with five or more progeny having herdmates. A total of 1,262,814 progeny were included in the genetic appraisals. (AH i4-1)

2. Cow evaluation program. Genetic appraisals of the registered cows in DHIA were made and the resulting indexes of performance made available to the industry on a semiannual basis. A total of 550,000 cows were indexed biometrically and the top 2% identified. This indexing procedure provides the industry with a reliable and uniformly derived estimate of breeding value. These sire and cow evaluations will be especially adaptable to genetic improvement through artificial insemination. (AH i4-1)

3. Dairy recordkeeping programs. The 1,424 dairy herd improvement associations, employing 2,439 supervisors, provide the organizational structure for visiting farms and for reporting data in the 50 cooperating States. Participation in the National Cooperative Dairy Herd Improvement Program continued to expand and was as follows:

<u>Plans</u>	<u>Herds</u>	<u>Cows</u>
Standard DHIA	40,075	2,087,581
Owner-Sampler	26,604	818,406
Weigh-a-Day-a-Month	<u>1,229</u>	<u>56,570</u>
Total	67,908	2,962,557

A total of 1,685,797 records of performance was reported to the Dairy Cattle Research Branch for use in the genetic appraisal of cows and sires and for research.

The artificial breeding program, through which the superior sires developed and recognized in DHIA herds are utilized, bred a total of 7,282,994 dairy and 464,959 beef cows. This represents 41% of the Nation's dairy cows of breeding age. An average of 3,053 cows per sire were inseminated during the year. The total of 7,747,953 cows were inseminated by 2,538 bulls. (AH i4-2)

#### 4. DHIA record analysis and research.

(a) Recordkeeping statistics. Cows in standard DHIA herds produced 11,976 pounds of milk and 457 pounds of milk-fat per cow-year in 1964-65, and exceeded cows not enrolled in recordkeeping by 4,708 pounds of milk. The rate of yearly increase in milk yield of DHIA cows during the past 3 years has been 98% more rapid than for non-DHIA cows.

Cows in DHIA continue to be fed higher levels of feed, and provided an estimated income over feed cost of \$295 per cow. This return compares with an estimated income over feed cost of \$284 in 1963-64; \$279 in 1962-63; and \$295 in 1961-62. (AH i4-3)

(b) Reliability of early sire evaluations. An analysis of 277 heavily tested AI bulls was made in order to determine the frequency, degree, and source of negative bias in sire summaries. A low level bias was found, and its removal affected by implementing a procedure which will delay the use of lactation records until the majority of progeny calving at the same time are available. It was also found that the first AI daughters of a bull are representative of his transmitting ability for production. (AH i4-3)

(c) DHIA 305-day projection factors. A set of multiplicative factors were developed for nationwide use in further standardizing DHIA lactation records for cow-days in milk. It was necessary to develop and use a total of 6,960 factors, one for each breed, day in milk (15 to 304), age group, and milk and milk-fat yield. These factors will significantly increase the usefulness of all DHIA records of performance, both on-the-farm and in research studies. (AH i4-3)

(d) Evaluation of age correction factors. A study of the paired and gross comparison method of determining age correction factors was made using production data grouped by region. The deviation between gross and paired regressions on age was found to be noticeable only for the Western Midwest and Plains areas. In most instances, however, and particularly at ages beyond maturity, the standard and presently used DHIA age factors offer a reasonable compromise between those derived by the gross and paired methods. (AH i4-3)



(e) Usefulness of records of dams in the evaluation of sires in artificial insemination (AI). DHIA data representing 24,853 daughter-dam comparisons for 263 Holstein AI sires were studied to determine if the use of data from dams (mates of sires) will increase the reliability of a sire's evaluation. The relative ranking of the bulls was the same (rank correlation = + .998) when daughters' deviations alone were used, as when both daughter and dam deviations were employed for ranking; although there was a mean bias of 41 pounds of milk per sire due to selection of mates. These results demonstrate that dam-herd mate comparisons need not be added to daughter-herdmate comparisons when genetically evaluating AI bulls. (AH i4-3)

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##### Dairy Cattle

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INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 29.0 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis of Cattle 2.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to \$31,950.00 over a 3-year period.

Vibriosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and through a contract with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 3.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado State University at Fort Collins, the Agricultural Experiment Station, Purdue University at Lafayette, Indiana, and the Iowa State University, Ames.



Mastitis of Cattle 3.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease of Cattle (Shipping Fever) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than Vibriosis and Trichomoniasis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Foot Rot (Infectious Pododermatitis) of Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization against Bovine Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Enteritis of Young Calves 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a contract with the University of Idaho, Moscow.

Bovine Lymphosarcoma 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis of Cattle (Johne's Disease) 2.0 at the National Animal Disease Laboratory, Ames, Iowa

Keratitis (Pink Eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

#### PROGRAM OF STATE EXPERIMENT STATIONS

The State experiment stations are devoting an increasing amount of effort toward research directed at the prevention, control and eradication of cattle diseases. The objectives of these studies are concerned not only with more efficient production of meat and milk, but also with the production of products which are wholesome and safe for human consumption.

Many of the Western states are cooperating (W-88, Enteric Diseases of Neonatal Calves) in initiating basic and applied studies to determine the cause and control of intestinal infections in calves.

Increasing interest and support is being given to the cause and control of bovine leukosis. Workers also seek basic information concerning the possible relationship of this disease to cancer in man and other animals.

Cooperative regional studies, among the Southern (S-30, Diseases of Reproduction) and Northeastern states (NE-40, Pathology of Breeding Failure), seek to determine the relation of various infectious agents to poor reproductive performance and sterility in cattle. Increasing attention is being directed toward the role of viruses in infertility. A new vaccine against vibriosis offers promise. However, antigenic variation in strains of the organism causes continued concern to workers. The role of leptospira in infertility is being determined. Basic studies pertaining to the diagnosis and pathogenicity of the different serotypes of the organism continues to receive considerable attention.

The North Central states are cooperating (NCR-29, Shipping Fever of Cattle; NCR-37, Mucosal Disease of Cattle) to determine the inter-relationships between various agents and factors associated with respiratory infections in cattle. The relation of virus diarrhea and infectious bovine rhinotracheitis to the respiratory disease complex is being given considerable attention. Preventive vaccines are being developed and evaluated under laboratory and field conditions.

Many of the states, particularly those in the north central region, (NCR-47, Mastitis in Cattle) are cooperating informally in seeking to determine the cause and effective methods of controlling mastitis in cattle. Preventive and therapeutic agents are being evaluated to determine their efficacy. Residue studies are an important part of these investigations.

Attempts are being made to determine the role of certain viral agents in foot rot and infectious keratitis (pink eye).

Much effort is being made to develop means of controlling urinary calculi in cattle (Regional Research project, W-41, Urinary Calculi of Cattle). Consideration is being given to the theory that an imbalance of certain nutritional elements may contribute to the development of the condition.

Sporadic diseases and new problems not previously encountered often become economically important enough to require intensive investigation. Other bovine disease conditions currently under investigation include epizootic bovine abortion, toxicoses, ketosis, parturient paresis, white muscle disease, aplastic anemia, enterotoxemia, porphyria, tuberculosis, paratuberculosis, various abnormalities, bloat, brucellosis, Q fever, etc.



Increased attention is being paid to public health aspects of animal disease research. Greater emphasis is being placed on research which would control and eradicate animal diseases transmissible to man. The protection of the consumer against foodborne diseases is also receiving considerable support.

The total State scientific effort devoted to diseases of cattle is 132.1 professional man-years.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Brucellosis of Cattle

In a research study conducted at the National Animal Disease Laboratory (NADL), Ames, Iowa, fifteen bulls from 4 to 10 months old were vaccinated against brucellosis to determine the nature and frequency of persistence of Brucella abortus Strain 19. A mild transient postvaccinal orchitis was detected in 8 bulls. Postvaccinal brucellemia was detected in 13 of the 15 bulls and persisted up to 28 days in one bull. Semen samples collected from the bulls had no noticeable decrease in quality and did not contain Brucella. Postvaccinal serum agglutinin titers persisted longer at diagnostic levels in bulls than in heifers vaccinated at the same age in a previous experiment. Ten of the 15 bulls still had "reactor" or "suspect" titers at 18 months of age.

The bulls were killed at 18 months of age and at necropsy there was no evidence of gross or microscopic pathologic alterations attributable to Strain 19, nor could the organism be demonstrated in any of the tissues. Under the conditions of this experiment Strain 19 did not localize or persist in any of the 15 bulls. However, persistence of serum agglutinin titers in bulls of breeding age may confuse interpretation of test results and hinder the progress of brucellosis eradication. (NADL) (ADP al-3(R))

The University of Minnesota, under a cooperative agreement with the USDA, reported finding that the number of animals in the herd influences the efficiency and sensitivity of the Brucella Ring Test (BRT). Work is being continued on the development of improved methods for conducting the BRT to increase or decrease the sensitivity according to the number of animals in the herd. The results will greatly increase the efficiency and applicability of the BRT as a screening test for Brucella agglutinins in milk and increase the usefulness of the test in accordance with the increasing size of herds to meet economic and technologic change. (Minnesota) (ADP al-3(R))

Ohio Agricultural Experiment Station, Wooster, under a cooperative agreement with the USDA, reported progress has been made on the first and second phases of the program on serological tests and vaccination of heifer calves at 2 and 3 months of age respectively, with Brucella abortus, Strain 19 vaccine. Some of the heifers 15 to 18 months of age have been bred.

(Ohio) (ADP al-3(R))

The University of Wisconsin, Madison, under a cooperative agreement with the USDA, reported finding a standardized complement fixation test, used in the diagnosis of bovine brucellosis on over 60,000 serum samples during the past three years, has been the most effective of the supplementary serological tests for the detection of infected animals regardless of the tube agglutination test titer. In no case was B. abortus isolated from an animal with a CF titer of less than + at 1:20. Over 90% of proved infected animals had CF titers of + at 1:40 or higher.

Cultures indistinguishable from Strain 19 in CO<sub>2</sub> independence, sensitivity to thionin blue, safranin O erythritol and penicillin have been isolated from 27 cows in Wisconsin, one from Virginia, one from Washington, one from Israel, and seven from New York over the past three years. Ten of these have also been found indistinguishable from Strain 19 in virulence for guinea pigs. These are interpreted as being authentic cases of persistent infection with Strain 19.

A method of dissociating antibody from brucella antigen in tissue by treatment with SM urea has been developed. This has been adapted to improvement of fluorescent antibody staining of Brucella abortus in tissue and to the proving of specificity of staining of antigen in a single preparation. The method should have general application in the fluorescent antibody technique.  
(Wisconsin) (ADP al-3(R))

#### B. Vibriosis

The New York Veterinary College, Cornell University at Ithaca, under a cooperative agreement with the USDA, reported the following:

Diagnosis of Vibriosis in the Bull by use of the Fluorescent Antibody Technique. A fluorescent antibody conjugate was prepared by labelling the gamma globulin fraction from a pool of rabbit antisera for one strain of Vibrio fetus venerealis with fluorescein isothiocyanate. Nonspecific fluorescence in stained specimens was minimized by using a fraction of the conjugate separate by ion-exchange chromatography. Cross reactions in the fluorescent antibody reaction were observed with intestinal and venereal strains of V. fetus but not with V. bubulus or 17 other species of bacteria tested.

The conjugate was used to stain smears of preputial fluid from a group of 24 bulls. This group included known carriers and bulls of varying ages from which Vibrio fetus had not been isolated. Samples from each bull were examined weekly for 6 consecutive weeks. Complete agreement was obtained between the results of the fluorescent antibody tests on preputial fluid and the results of cultural examination of semen samples from these bulls. The results of this experiment indicate that the fluorescent antibody reaction provides a highly accurate and sensitive method for the detection of V. fetus carrier bulls.



Diagnosis of Vibriosis in the Bull by Isolation of the Organism from Semen. A six-months' study has been completed for the purpose of evaluating different cultural procedures on semen for diagnosing vibriosis and obtaining an estimate of the efficiency of semen culture and as a method for detecting V. fetus carrier bulls.

Five semen samples from each of 35 Vibrio fetus carrier bulls were cultured at monthly intervals. V. fetus was identified in 151 of the 175 samples (86.3%). Of 146 isolates fully characterized biochemically all were classified as Vibrio fetus venerealis. Isolations were made in 5 of 5 attempts from 17 bulls, 4 of 5 attempts from 12 bulls, and 3 of 5 attempts from 6 bulls. On the basis of these results it would be necessary to culture two or three semen samples in order to establish a bull's carrier status with confidence. The sensitivity of this method probably falls somewhat below that of fluorescent antibody tests on preputial scrapings. Direct culturing on antibiotic medium, in which spread plates were prepared from undiluted semen from three serial tenfold dilutions, was found to be a much more efficient method of isolating Vibrio fetus than was culturing of filtered samples.

(New York)

(ADP al-9(R))

### C. Tuberculosis

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa. The studies pertained to:

Glycoprotein Levels in Cattle Naturally and Experimentally Infected with Mycobacterium bovis. The study was to determine if serum glycoprotein changes which are used in the diagnosis of tuberculosis in other species might be applicable to the diagnosis of tuberculosis in cattle. In experimentally infected cattle there was a significant rise in the serum glycoprotein during the first five weeks after infection. After this the glycoprotein levels tended to decrease. No evidence was found correlating an increased serum glycoprotein level with either positive skin tests or the presence of lesions in naturally infected cattle. The test does not appear to be of value in the diagnosis of tuberculosis in cattle.

Concentration Effects in Cervical Tuberculin Tests of Cattle Naturally Infected with Mycobacterium paratuberculosis. In determining the effect of simultaneous intradermal cervical injections of two concentrations of ARS tuberculin on the intradermal reactions to each concentration, it was found that simultaneous injections at two concentrations did not significantly affect the size of reactions.

In determining if cattle react differently to the two concentrations, the reactions to full strength tuberculin were significantly larger than those to tuberculin diluted 1:10 whether read at 24, 48, 72, or 96 hours.

In comparing the accuracy of two methods of measuring the reactions, the interpretation of the reactions was the same whether measured by palpating or with a dermal thickness gauge.



An intravenous tuberculin test was used in 76 cattle to supplement but not replace the intradermal tuberculin test in detecting infected cattle which fail to react to the intradermal test. Forty-seven cattle reacted to the intradermal test of which 16 also showed substantial temperature increases with the intravenous test. Fourteen of the 16 had lesions of tuberculosis and 16 that did not show temperature increases also had lesions.

Twenty-nine cattle did not react to the intradermal test, 24 of these did not show temperature increases to the tuberculin administered intravenously. The remaining 5 did show temperature increases, and all 5 were found to have lesions of tuberculosis on postmortem examination.

(NADL)

(ADP al-13(R))

Research was continued at the Michigan State University, East Lansing, under two contracts with the USDA. Reports submitted are as follows:

Contract No. 12-14-100-6852(45). The final report on work under this contract was received during the fiscal year 1965. Findings were:-

Polysaccharide specific antibodies and phosphatide specific antibodies, as determined by the hemagglutination (HA) test and the kaolin-phosphatide (KP) test, were elicited in calves by experimental infections with Mycobacterium bovis and M. avium. At necropsy, the calves inoculated with M. bovis had lesions and progressive disease. The calf inoculated with M. avium had lesions and non-progressive disease.

Calves inoculated with pseudochrome and Group IV atypical mycobacteria did not have increased HA and KP serum titers. At necropsy, no lesions or disease were detected.

Calves inoculated with Group III atypical mycobacteria (bovine and porcine origin) had varying HA and KP serum titers. Group III organisms (bovine origin) had a range of virulence from none to that almost equal to M. bovis and those of porcine origin produced few or no lesions in calves. The HA and KP serum titers could not be consistently correlated with disease.

The cows from a gross-lesion herd had fourfold or greater increases in HA and KP serum titers after administration of tuberculin. Two cows from a no-gross-lesion herd did not exhibit the anamnestic-like response. At necropsy the former cows had lesions and progressive disease - the latter cows did not have lesions or disease.

Swine which were inoculated with M. bovis, M. avium, and Group III organisms (porcine origin) had lesions at necropsy and fourfold increases in HA and KP serum titers after administration of tuberculin.

Three of four calves, which were inoculated intradermally with heat-killed organisms, had fourfold increases in HA serum titers. None of the four calves had lesions or disease.



The route by which some of the Group III organisms (bovine origin) were administered to cattle altered the serologic response. When inoculated intradermally, all calves had a fourfold or greater increase after the first tuberculin test.

When the three strains of Group III organisms were introduced into the uterus, four of the nine heifers did not have a fourfold increase after the first tuberculin test.

When the three strains of Group III mycobacteria were administered in an aerosol, the HA serum titers were considerably lower.

Specific phosphatide extracts prepared from a representative group of known and atypical mycobacteria did not increase the specificity of the kaolin-phosphatide test.

Generally, swine and cattle with lesions exhibited the anamnestic-like response to the first tuberculin test.

Contract No. 12-14-100-7164(45). In Section I, the study relative to virulence of 12 cultures of microbacteria in calves, swine, guinea pigs, rabbits, and chickens, is approximately two-thirds completed. In Section II the study and comparison of the disease in adult cattle and calves by Group III microbacteria, is nearing completion. In Section III progress is being made in the studies designed to produce and evaluate sensitins for the detection and differential diagnosis of microbacterial infections in laboratory animals. In Section IVa. guinea pigs were inoculated intraperitoneally with M. bovis organisms. Serums were collected from them at intervals of 7 - 10 days post-inoculation. Ouchterlony immunodiffusion was not a satisfactory way to compare serums from normal and tuberculous guinea pigs. A progressive depletion of the  $\alpha_1$  globulin lipoproteins and a simultaneous increase in the slow moving  $\alpha_2$  globulin lipoproteins occurred in the serums from the tuberculous guinea pigs.

Hyper $\alpha_2$ -globulinemia was detected by cellulose acetate electrophoresis in the serums from tuberculous guinea pigs. Coincident was the detection by immuno-electrophoresis of an additional  $\alpha_2$  globulin, tentatively named  $\alpha_2$ -T. Cellulose acetate electrophoresis and immuno-electrophoresis of serums obtained from guinea pigs sensitized to tuberculin with heat-killed M. bovis revealed neither hyperalphaglobulinemia nor the  $\alpha_2$ -T.

Gel infiltration of normal serum in Sephadex G-100 and G-200 was not a satisfactory procedure for the separation of the serum antigens.

Column chromatography on DEAE-cellulose separated normal serum into four major and several minor fractions.



Normal serum was separated into three main fractions by electrophoresis in insoluble potato starch. Satisfactory separation of the serum antigens was not obtained by either of these procedures. Normal serum was separated into five fractions by electrophoresis in agar gel. From seven to nine fractions were readily resolved by electrophoresis on cellulose acetate membranes. Thirty antigens were found in normal serum by immuno-electrophoresis.

Interpretation of the different phases of the work under this contract will be submitted in the final report. (Michigan) (ADP al-13(R))

#### D. Mucosal-Respiratory Disease-Complex of Cattle

Research work was continued at the National Animal Disease Laboratory, Ames, Iowa. The report shows:

Studies on the intracellular synthesis, separation and characteristics of the soluble antigen of bovine viral diarrhea virus have been completed. Complement fixing soluble antigen was detectable intracellularly before the appearance of infectious virus during synthesis in roller flask cultures of bovine embryonic kidney cells. The release of infective virus into the extracellular fluid was concomitant with the release of soluble antigen.

Soluble antigen was separated from the infective virus. It was heat labile at 56 C, but stable in buffers at pH 5.0, 7.0, and 9.0 at 37C. It was irreversibly precipitated in buffers at pH 3.0 or below. Trypsin and a chymotrypsin completely inactivated the soluble antigen whereas ribonuclease and deoxyribonuclease had no detectable effect on the complement fixing activity. There was no apparent serologic relationship between the soluble antigen of bovine viral diarrhea virus and arbovirus group B and lymphocytic choriomeningitis virus antisera.

A strain of bovine viral diarrhea virus, NADL-MD, was adapted to primary and a cell line of swine kidney cell cultures.

Prior infection of the swine kidney cell line with modified hog cholera virus completely abolished the cytopathic effect and suppressed the yield of adapted NADL-MD virus. The interference occurred prior to the formation of soluble antigen since this was decreased two to fourfold and virus yield was decreased by 90 percent.

Application of the interference test made it possible to study the rate of development of neutralizing antibody against hog cholera virus.

A modified strain of hog cholera virus used to infect swine kidney cell cultures, interfered with the cytopathic effect and virus yield of adapted NADL-MD virus used for challenge. Interference by modified hog cholera virus was dosage dependent and required infection of the cells before interference was expressed. It was demonstrated that interferon prepared



by conventional methods played no role in interference. A swine-passaged strain of hog cholera virus did not produce as complete interference as the strain passaged in swine kidney cell cultures.

When cell cultures were experimentally infected with bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) viruses, the latter outgrew the former in relatively few passages, thus indicating that isolation of a mixed population of BVD and IBR viruses from an infected animal could possibly give rise to a pure culture of the latter virus in a relatively short time after the primary isolation.

Calves born to dams devoid of neutralizing antibody against bovine viral diarrhea virus (BVDV), although housed with other mature animals having antibody titers, have not become infected with the agent during a period of 8 months. When dams possessed neutralizing antibody against BVDV, it was transmitted to the calves at the same or higher titers but then decreased over the following 5 months until the serums became negative. In both of these examples the calves were kept with their dams and allowed to nurse. Results to date indicate that BVDV is not readily transmitted in the absence of clinical cases or other as yet unknown factors.

(NADL) (ADP al-14(C)(R)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, reported that the longevity of immunity of cattle to infectious bovine rhinotracheitis (IBR) has been found to be longer than 5½ years. The quantitative relationship between neutralizing titer and the susceptibility of the cattle will give a guide for vaccination and diagnostic purposes. IBR will produce abortion. (Colorado) ADP al-14(C)(R)

Research investigations were continued at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA. The report shows that the apparent incidence of the mucosal disease complex has not changed. Most cases tend to be typical of chronic cases of the syndrome earlier called "virus diarrhea." However, some typical acute outbreaks of "mucosal disease-Iowa" are encountered in the Lafayette area in which mortality approaches 100% of affected animals, and morbidity 15 to 20 percent.

The properties of two cytopathogenic strains of bovine viral diarrhea (BVD) virus, Indiana 1061, and reference strain Oregon C24V, were studied using tissue culture methods. In vitro virus assays were performed in bovine kidney (BK) cell cultures. Indiana 1061 strain virus was isolated from the spleen of a calf in which pathological lesions of BVD were found at necropsy. A mild disease syndrome, indistinguishable from experimental BVD, was observed in calves that were inoculated with spleen suspension. Reciprocal cross-immunity tests demonstrated that calves immunized against Indiana 46 strain BVD virus were resistant to challenge with the new isolant. Calves that had been inoculated with spleen suspension did not react to a challenge injection of Indiana 46 strain virus.



Serum neutralization tests indicated that the newly isolated virus, designated Indiana 1061, was antigenically related to reference strain Oregon C24V. Serums obtained from calves following inoculation with Indiana 1061 and Oregon C24V viruses neutralized the cytopathic effects of Indiana 1061 virus in BK cell cultures.

Bovine kidney cell cultures infected with Indiana 1061 or Oregon C24V viruses, did not absorb guinea pig, bovine or ovine erythrocytes when incubated at 25 C. or 4 C. Erythrocytes added to the culture medium were not agglutinated.

Morphologic changes occurring in BK cells following inoculation with Oregon C24V virus showed the initial degenerative changes were confined to the cytoplasm of the cells and were characterized by condensation and vacuolization of the cytoplasm, followed by obvious nuclear changes. Cavitations in the nucleoli occurred early in the course of degeneration.

Cytochemical staining with acridine orange revealed an increase in cytoplasmic and nucleolar fluorescence (ribonucleic acid) (RNA) at 24 hours. As progressive infection of the cell sheet occurred, cytoplasmic (RNA) fluorescence increased. Treatment of cultures with RNAase completely removed the cytoplasmic and nucleolar staining.

Anti-Oregon C24V fluorescein labeled globulin specifically stained cultures infected with Indiana 1061 or Oregon C24V viruses. Specific fluorescent staining was detectable 16 hours after inoculation and prior to the development of cytopathic changes. Viral antigen was found diffusely spread throughout the cytoplasm, and in some cells was concentrated in a perinuclear location. "Rounded-up" cells showed brilliant fluorescent staining. Large particles or chunks of fluorescing material could frequently be seen in the cytoplasm of degenerating cells. In vacuolated cells, the viral antigen was concentrated at the periphery of the vacuoles. Viral antigen was not observed in the nucleus of cells. Fluorescent staining was completely or markedly inhibited when homologous immune globulin was mixed with conjugates prior to staining.

The Specific-Pathogen-Free (S.P.F.) cattle herd continues to be relatively free of important pathogens with the exception of serological evidence of parainfluenza (SF-4). Titers were highest in animals under one year but colostrum-deprived calves had no titers. The reproductive efficiency of the herd is normal and about 20 calves will be available for research during the next 12 months. (Indiana) (ADP al-14(C)(R)

Iowa State University, Ames, under a cooperative agreement with the USDA, reported the direct fluorescent antibody (FA) test is well suited to the diagnosis of cases of infectious bovine rhinotracheitis (IBR). The method appears to be more trustworthy than isolation.



Immunological tolerance studies with bovine virus diarrhea (BVD) infected calf fetuses have been initiated with the injection of bovine fetuses in the first and second trimester of fetal life. The injection of virulent virus apparently does not cause abortion, or elicit an antibody response in the dam. Calves will be recovered by caesarean section and raised in isolation for three months prior to challenge.

Four strains of IBR are being studied in an attempt to associate basic properties with observed differences in pathogenicity. Although these strains will cross neutralize in standard neutralization tests, kinetic neutralization studies show some differences between strains.

(Iowa) (ADP al-14(C)(R))

#### E. Mastitis of Cattle

The research studies at the National Animal Disease Laboratory, Ames, Iowa, related to the following:

The nonhemolytic and weakly hemolytic coagulase-negative staphylococci, known as Staphylococcus epidermidis, have been considered nonpathogenic and frequently neglected as a cause of bovine mastitis. The organisms are not typable by bacteriophage and do not produce toxins. Methods for characterizing and differentiating these organisms are needed for enzootiological studies. A study was made to determine whether the various degrees of pigmentation might be useful in differentiating strains. Spectrophotometric analysis of the pigments of 70 isolates, representing 48 strains of Staphylococcus epidermidis, exhibited absorption curves that were classified into seven types, designated I, II, III, IV, V, VI, and a S. aureus type. Two subtypes were included in types I and III. All non-pigmented cell extracts were classified as type I. Three isolates gave an absorption curve that was similar to the curve produced by extracts of five of seven S. aureus strains, thus the designation S. aureus type. The differences in pigment complexes indicated by the various absorption curves of methanol extracts were substantiated by column-chromatography studies. Generally, pigments of types II to VI, as produced by representative strains, were of a xanthophylllic nature, i.e., more soluble in methanol. The S. aureus type pigment studied was carotenelike, i.e., more soluble in petroleum ether. Analyses of representative strains showed that the type of spectral absorption curves did not change whether the organisms were carried in vitro and tested through 3 months, or isolated repeatedly from infected udders for periods up to 8 months. The method of determining the spectral absorption curves of whole-cell methanol extracts provides an additional tool for differentiating strains of S. epidermidis that can be used in enzootiological studies of udder infections.

(NADL) (ADP al-15)



The University of California, Davis, under a cooperative agreement with the USDA, submitted a report referring to previous studies that indicated the important role of preleukocytosis (inflammatory response) in protection of the lactating bovine mammary gland against peracute coliform mastitis. Acute mastitis develops when the inflammatory reaction (leukocytes specifically) destroy the flora releasing the endotoxin. High molecular weight polysaccharides of bacterial origin have been tried as a method to prevent diapedesis of leukocytes. Commercially prepared dextran (PHARMACIA Chemicals, Uppsala, Sweden), in increasing amounts and of higher molecular weight than reported as an effective agent, gave no difference in either the speed or magnitude of the leukocytic infiltration in experimentally infected mammary glands than in glands of control cows receiving no dextran. Bacterial levans of higher molecular weight were found to be more effective than dextran in laboratory animals.

Aerobacter levanicum levan was prepared and injected into a cow prior to inoculation of a gland with Aerobacter aerogenes. The injection proved to be fatal to the cow presumably due to an overdose of the levan even though on a weight basis; less levan was injected than was the dextran. The effect resembled that of massive histamine release. No leukocytosis into the inoculated quarter took place during the 8 hours of survival post levan while the control cow developed the typical local reaction within 4 hours.

Several of the cows are now in their fifth lactation. No mammary gland infections with pathogenic staphylococci have appeared in this herd. This is evidence that staphylococcal mammary infections are not the inevitable consequence of aging and wear and tear of the milking act. The majority of these cows, although having experienced many episodes of acute or chronic experimental mastitis, secrete essentially normal, cell-free milk again within a short period of time after the pathogenic bacteria disappear.

Several natural, chronic infections with intermediate coliform organisms and paracolon-like organisms have occurred in some quarters of these cows. These infections have been persistent and have stimulated significant leukocytic infiltrations into the milk ( $\pm 5.0$  millions of cells/ml). Advantage has been taken of this to attempt to superimpose Streptococcus agalactiae or Pseudomonas aeruginosa in repeated daily doses without success. The pre-leukocytosis of  $\pm 5.0$  million cells prevented the inoculated organisms from multiplying and thus infection was precluded. Daily inoculation of Streptococcus agalactiae or Pseudomonas aeruginosa into glands having undulating levels of leukocytic infiltration have led to infection becoming established when infiltrating leukocyte level became reduced temporarily.

Pseudomonas aeruginosa appeared to be able to establish itself when multiple doses were given in the presence of infiltrating cells at  $\pm 1,000,000$ /ml. of foremilk. Str. agalactiae even in doses of several thousand was held in check by a pre-existing inflammatory response characterized by foremilk cell counts of 500,000. Commonly, cell numbers/ml. in strippings milk are



several fold greater than in foremilk. Strippings counts of several million in the face of foremilk cell numbers of less than 1,000,000 appear to reflect a considerable barrier to both Str. agalactiae and coliform bacteria.

Cultures of fresh milk fail to demonstrate the pseudomonads. Pre-incubation of milk is required for colony growth on culture from chronically infected lactating glands. The Pseudomonas aeruginosa organisms readily grow on culture of fresh fluids drawn from chronically infected dry glands.

Studies are demonstrating the effectiveness of pre-existing leukocytosis in preventing establishment of Str. agalactiae and Ps. aeruginosa when entering the gland in small numbers as a single incident.

(California) (ADP al-15)

#### F. Respiratory Diseases of Cattle (Shipping Fever)

Research investigations conducted at the National Animal Disease Laboratory, Ames, Iowa, are being continued on basic studies on the physiology of organisms associated with shipping fever. A semi-defined medium for the growth of Pasteurella haemolytica was developed. It consisted of acid-hydrolyzed casein, supplementary cysteine, inorganic salts, and either D-galactose or sucrose as the carbon source. Essential vitamins were pantothenic acid, nicotinamide, and thiamine. The phosphorylated forms of thiamine were more efficient than thiamine itself in promoting growth. Six strains of P. haemolytica, isolated from cases of bovine respiratory disease, grew well in the medium. The medium is being used in studies on the effect of bovine tissue exudates and fluids upon the nutrition and metabolism of Pasteurella species.

Exposure studies in specific pathogen free calves using parainfluenza-3 virus and Pasteurella haemolytica, singly or in combination, produced a clinical syndrome closely resembling "shipping fever."

(NADL) (ADP al-17)

#### G. Etiology of Infertility in Cattle other than by Vibriosis and Trichomoniasis

In research studies at the National Animal Disease Laboratory, Ames, Iowa, Mycoplasma was isolated, for the first time, from an aborted bovine fetus and from vaginal mucus of cattle with signs of infertility. Morphological and biochemical comparisons were made between the fetal and vaginal strains and 4 strains from other sources. The fetal isolant differed from others in colonial morphology, methylene blue reduction and carbohydrate fermentation. Vaginal isolants were similar in colonial morphology and biochemical properties with the exception of serum requirement. Similarities were also noted between the bovine and non-bovine strains.

Additional work is needed to determine the serological relationship of the fetal and vaginal isolates as well as the importance of Mycoplasma as a cause of bovine infertility. (NADL) (ADP al-19)

#### H. Epizootic Bovine Abortion

The University of California, Davis, under a cooperative agreement with the USDA, reported that previous studies indicated the failure of vaccination to prevent epizootic bovine abortion (EBA) might be because the cattle were vaccinated too near the time of their exposure to the causative agent to allow for the development of a protective immunity. Multiple injections of vaccine, beginning in calfhood and continuing to or following conception, is the present program.

A viable and an inactivated vaccine are being tested in the study. The antibody response has been exceptionally good, but the immunity in these cattle has not been challenged with virulent virus as yet.

Indications are that the EBA virus is becoming attenuated by continued serial passage in chicken embryos and mice.

Preliminary findings indicate that antibiotic therapy might be of some value in controlling EBA when treatment is initiated just before or following exposure to the virus.

It was shown conclusively that the EBA agent is not spread by venereal means as once believed. (California) (ADP al-21)

#### I. Immunization Against Bovine Leptospirosis

Work at the National Animal Disease Laboratory, Ames, Iowa, shows that bovine leptospira were grown in chemically characterized medium free of serum or serum protein, but the leptospiral growth was poor and the organisms lacked certain surface antigens which are believed to be involved in immunity. The addition of rabbit serum or bovine albumin to cultures of L. pomona in synthetic medium did not restore their antigenicity. It was restored after a minimum of seven generations in medium supplemented with rabbit serum. Various synthetic peptides or fragments of albumin did not replace albumin. The end groups of the albumin molecule were not essential for its function. (NADL) (ADP al-25)

#### J. Chemotherapy in Leptospirosis

This work is being conducted at the National Animal Disease Laboratory, Ames, Iowa. The minimal, growth-inhibitory concentrations ( $\mu\text{g./ml.}$ ) of antimicrobial agents and dyes for Leptospira pomona, Leptospira canicola, Leptospira autumnalis, and Leptospira grippotyphosa in synthetic medium were: penicillin G, 0.06; oxytetracycline and chlortetracycline, 0.5; erythromycin, 0.025; tylosin, 0.05; actinospectacin and kanamycin, 0.25;



dihydrostreptomycin, 0.3; chelocardin and lincomycin, 2.0; fucidin, capreomycin, isoniazide, and chloramphenicol, 20.0; sulfachlorpyridazine, sulfamethoxazole, and sulfadimethoxane, 1000; crystal violet, methylene blue, and pyronin B, 0.2; and thionin and basic fuchsin, 20. Semi-synthetic penicillins (oxacillin, methicillin, and phenathicillin) were five to fifty times less effective than penicillin G. Tylosin, kanamycin, tetracyclines, and penicillins were two to five times less effective in medium supplemented with rabbit serum than in synthetic medium. No differences in sensitivity to dihydrostreptomycin were found among 12 leptospiral serotypes including virulent strains and nutritionally fastidious strains. Dihydrostreptomycin, chlortetracycline, and penicillin at high concentrations affected leptospiral respiration, motility, and viability differently. Dihydrostreptomycin had little effect on the respiration or motility of L. pomona, but subcultures failed to grow; chlortetracycline, but not penicillin, rapidly inhibited all three parameters.

Chemotherapeutic agents, which inhibited leptospiral growth in vitro, were administered to hamsters in attempts to eradicate renal L. pomona. L. pomona was cultured from only one of 50 infected hamsters treated with dihydrostreptomycin (25 mg/kg once daily for 3 days).

The following drugs (mg./kg. of body weight once daily for 3 days) were not effective: crystal violet, methylene blue, pyronin B (25); penicillin G (30 and 60); phenathicillin, oxacillin, and methacillin (25); fucidin, kanamycin, nalidixic acid, and capreomycin (25); lincomycin, chelocardin, chlortetracycline, oxytetracycline, tetracycline, chloramphenicol, actinospectacin, tylosin, and erythromycin (12.5, 25, and 50).

Neither chlortetracycline in the feed (1,000 Gm./ton for 10 days) nor dihydrostreptomycin in the drinking water (25 mg./kg. for 7 days) eliminated renal leptospires. (NADL) (ADP al-26)

#### K. Investigations on Bovine Lymphosarcoma

The report from the National Animal Disease Laboratory, Ames, Iowa, shows that in the investigation of field cases of bovine lymphosarcoma, a blood protozoan parasite tentatively identified as Trypanosoma theileri, has been isolated from the leukocytes in all cases. The parasites can be detected in leukocyte cultures from 1 to 6 days after the cells have been planted. It appears essential that fetal calf serum should be used in the medium rather than serum from the affected animal in order to isolate the parasites consistently. Serum from the affected animal causes the parasite to disappear from the cultures and this may be due to antibodies. Relationship of the parasite to the cause of bovine lymphosarcoma has not been determined. (NADL) (ADP al-30)

## L. Paratuberculosis (Johne's Disease) of Cattle

Reports from the National Animal Disease Laboratory, Ames, Iowa, summarizing the work of several years, show there is no known satisfactory method of diagnosing Johne's disease in carrier animals that are not showing clinical signs of the disease.

A herd of cattle, affected by the disease, consisting of approximately 175 animals, was studied for 6 years. Blood samples were obtained from all cattle twice a year and selected tissues of all cattle slaughtered were examined. Of 159 cattle removed from the herd and slaughtered during the study, 111 had high hemagglutination test titers (1:32 or higher). At slaughter the bacillus was harbored by 45 of these of which 22 were showing clinical signs of the disease. Sixteen of the remaining 48 with low hemagglutination titers (1:16 or less), also harbored the bacillus at slaughter and 4 of these had clinical signs of disease. From these results it appears that high hemagglutination titers do not seem to be closely enough associated with active disease to use this test for diagnostic purposes on individual cattle.

Ninety-eight adult cattle from herds infected with Johne's disease were tested by injecting johnin into the jugular vein. Rectal temperatures were recorded just before the injection and at set intervals after injection. A temperature rise of 1.5° F or more over the preinjection temperature, provided the highest temperature exceeded 103.2°F, was considered a significant reaction.

All cattle were slaughtered and examined culturally and microscopically for Johne's disease. On the basis of postmortem findings, they were classified as negative, lightly infected or heavily infected. Eighty percent of the heavily infected and 35 percent of the lightly infected cattle reacted significantly to the test. The remainder did not. The intravenous johnin test appears to have value as a diagnostic test as it has better correlation with postmortem findings than any other test now in use.

(NADL)

(ADP al-35)

## M. Infectious Keratitis (Pink-eye) of Cattle

At the National Animal Disease Laboratory, Ames, Iowa, in a series of preliminary experiments on bovine pink-eye, workers observed that a mercury sunlamp enhanced the effect of Moraxella bovis infection upon the bovine eye. The resulting disease was indistinguishable from field cases of infectious bovine keratoconjunctivitis (pink-eye). This method made possible the study of the disease under controlled conditions at any time of year. The workers proposed that ultraviolet has a primary etiological role in the disease. Work is being continued. Studies on the etiology of bovine pink-eye have been completed on the isolation and characterization of Moraxella bovis.

(NADL)

(ADP al-37)



N. The Immunizing Effect of Brucella Cell Wall (PL 480 project)

Under a PL 480 Grant, investigations on "The Immunizing Effect of Brucella Cell Wall" are in progress at the Hebrew University, Hadassah Medical School, Jerusalem, Israel. Their report shows that killed preparations derived from Br. abortus cell walls conferred immunity to mice for up to 90 days. The period following challenge during which the mice were free from infection was very short. Animals examined at 28 days or more after challenge with high doses of bacteria were found to harbor large numbers of Brucella organisms in the spleen. Neither reinfection from extraneous sites nor the multiplication of a small number of organisms in the spleen could be implicated in the phenomenon of reversion. Vaccines prepared from cell walls of Br. abortus, melitensis and suis proved more effective than intact cells. The more recent experiments with cell wall fractions indicate that further purification of the immunizing antigen is feasible.

(Israel) (A10-ADP-6)

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FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS  
DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of foot-and-mouth disease was in 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal disease foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25% in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90% or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continues, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.



## USDA AND COOPERATIVE PROGRAM

The Department at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on foot-and-mouth disease vaccines which is conducted cooperatively in The Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Assistance In Development Program, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The Federal scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 23.5 professional man-years. This effort is divided among sub-headings as follows:

Studies on foot-and-mouth disease virus 2.0

Determine mechanism of antibody formation 1.0

Immune response of cattle to types and sub-types of foot-and-mouth disease virus 1.0

Quantity production of foot-and-mouth disease virus 2.0

Establishment and characterization of cell lines and cell strains 1.0

Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells 2.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environment on foot-and-mouth disease virus 1.0

Bulk freeze drying of foot-and-mouth disease virus vaccine and antiserum 1.0

Identification, purification and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses 2.0

Immuno-chemical investigations of foot-and-mouth disease virus 1.5

Attenuation of representative types of foot-and-mouth disease virus 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanism of natural resistance and susceptibility to foot-and-mouth disease virus 1.0

Biological alteration of foot-and-mouth disease virus from continual residence in cell cultures 1.0

Morphological aspects of virus-cell relationships 1.0

Diagnostic and immunizing procedures for contagious bovine pleuropneumonia 3.0

Work was continued under a PL 480 grant to the Instituto Biologica, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

Under a PL 480 grant to the Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey, research is under way on "Studies of Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of Foot-and-Mouth Disease in Turkey."

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Immunological Studies on Foot-and-Mouth Disease Vaccine

Chemically treated baby hamster kidney (BHK) cell culture virus used with an oil adjuvant protected steers from infection with homologous virus exposure for a six month period. The response to this inoculum in cattle, appeared to correlate the response in swine.

Foot-and-mouth disease virus degraded to small particle by acid at pH 4.8 was found to react with foot-and-mouth disease (FMD) antibody and block it to the extent it was no longer able to neutralize infectious virus. This blocking could occur with heterologous small particle (type O) as well as the homologous type (type A). These data indicate strongly that the small particle of foot-and-mouth disease virus (FMDV) may have antigens common to all of the virus types and that specificity is associated with the intact virus particle. (ADP a8-8(R))

##### B. Immunological Study - Mechanism of Antibody Formation

The interaction between virus and antibody was found to be time and temperature dependent. These variables may reflect differences in neutralization values as determined by suckling mice and bovine kidney cell culture assays. Higher  $PD_{50}$  values were obtained at room temperature and at 37°C than at 0-5°C over a 6-hour period of incubation. Un-neutralized, infectious virus was detected in bovine kidney cell cultures from serum-virus mixtures that were innocuous for suckling mice. This observation may also account for the discrepancy between suckling mice and tissue culture assays.



Glycidaldehyde (GDA) was determined to possess desirable stability characteristics as compared to more popular inactivants. Little loss in virucidal potency was noted over a seven month period and after storage at ambient temperatures. A substantial amount of the virus inactivating capacity of GDA remains intact, although reduced, even after 24 hours at 37°C, while after 4 hours at 37°C, the slope of the inactivation curve was similar to that produced by freshly added GDA. A more rapid rate of inactivation by .02% GDA was noted when medium containing less GDA combining constituents was used. The overall effect of extraneous materials in the medium is to lower the concentration of GDA available for virucidal action and to decrease the rate of inactivation.

Suspensions of FMDv, A-119, propagated in baby hamster kidney cells, were treated with .02% GDA for 4 hours at 37°C. The rate of inactivation indicated a departure from first-order reaction kinetics. Two phases of inactivation were noted, but it is concluded that, with appropriate interpretation of the reaction kinetics, the inactivation does lend itself to that type from which a predictable margin of safety may be determined.

Infectious and killed virus preparations were antigenically potent and produced antibody levels capable of protecting animals against high challenge doses of infectious virus. (ADP a8-10(R))

#### C. Immune Response of Cattle to Types and Sub-Types of Foot-and-Mouth Disease Virus

Convalescent bovine sera have been assayed for neutralizing antibody, using suckling mice and tissue culture as the assay system. Values for levels of neutralizing antibody have been consistently higher when assayed in mice than when assayed in tissue culture. Differences in the rate of inactivation of virus by sera taken at different times following infection, appears to be attributable to the type of antibody present in the serum.

Knowledge of the protection conferred by foot-and-mouth disease vaccines and understanding of available methods for evaluating the degree and duration of such immunity are essential for planning of field vaccination programs. Information can only be obtained by observations and study involving large numbers of cattle over long periods of time. An ADP representative has been conducting investigations in cooperation with Dutch Ministry of Agriculture scientists in The Netherlands where annual vaccination of cattle against types O, A, and C foot-and-mouth disease is practiced. Cattle for serological and infectivity studies are available from selected Dutch dairy herds, from certain groups of animals destined for slaughter at the Amsterdam abattoir and animals used for vaccine potency tests at the Central Veterinary Institute.

Serum antibody titers against the three types of virus persisted over a four-year period in most of the 360 cattle which had been vaccinated two or more times at annual intervals. Antibody levels remained relatively

constant from 12 or 18 months following vaccination through a four-year period. Average antibody levels against type C virus were considerably lower than against types O and A.

In general, a good correlation was observed between serum antibody level and resistance to infection. In studies using type C virus, animals challenged two weeks following vaccination were more resistant than those with comparable titers exposed 9 to 48 months post-vaccination. (ADP a8-11(R))

#### D. Quantity Production of Foot-and-Mouth Disease Virus

Studies were continued to develop basic information on virus-cell inter-relationships applicable to better methods for detection, assay, and production of virus in cells grown on glass and in suspension.

Strains of all 7 types of foot-and-mouth disease virus (FMDV) were characterized according to frequency distribution of plaque diameters. There was a significant relationship between plaque size in primary cultures of bovine kidney cells and infectivity for cattle by intramuscular (i.m.) inoculation. Low dosage levels of large plaque strains were superior to small plaque strains for infecting cattle by the i.m. route. No relationship existed between plaque size and infectivity when the same strains were inoculated into mice, guinea pigs, cattle, or cell cultures. An inverse relationship existed between number of plaques formed by FMDV in primary bovine kidney cell cultures, and the concentration of bovine, young calf, or agamma newborn calf serum was used in the growth medium. Fewer plaques were formed when change medium contained bovine serum.

There was no evidence of reduced susceptibility of primary cultures of bovine kidney cells to infection with FMDV when serum containing antibodies against FMDV was used in the growth medium, providing the cell cultures were washed at time of medium change and the change medium was free of serum. Failure to wash the cultures resulted in reduction of number of plaques in cultures inoculated with virus homologous to the serum. (ADP-a8-12(R))

#### E. Establishment and Characterization of Cell Lines and Cell Strains

Plaque formation by different strains of FMDV in a line of baby hamster kidney cells is being studied.

Studies on susceptibility of a line of pig kidney cells to infection with various strains of FMDV have been initiated. (ADP a8-14(R))



## F. Mechanism of the Interaction between Foot-and-Mouth Disease Virus Molecules and Host Cells

Chemically-defined medium for baby hamster kidney cells (BHK). Studies were made on the development of a protein-free, chemically-characterized medium for the growth of baby hamster kidney culture cells on glass for use in the study of the biosynthesis of FMDV. In high multiplicity infection experiments in BHK cells of 5-hour duration, no definite medium was as satisfactory as the original growth medium containing serum and lactalbumin hydrolyzate. TB-H, glu, fortified with 0.03% glutamine, allowed the production of 0.5 log units less virus infectivity than complete growth medium, and was the best defined medium to be developed thus far. In media studied, the virus produced within 5 hours was still largely intra-cellular. Glutamine was not replaceable by lactalbumin hydrolyzate, nor by substances known to be produced from it in vivo, i.e., glutamic acid, proline, aspartic acid, asparagine, and the RNA bases uracil, cytosine, adenine, and guanidine. Glutamine stimulated virus production independently of the action of glucose, each chemical yielding about 1.2 log units less virus than complete growth medium. Glutamine, unlike glucose, stimulated oxygen uptake in uninfected cells by an average of 23%. In infected cells, which showed decrease in oxygen uptake, glutamine was able to enhance respiration. This apparent maintenance of cellular viability by glutamine may help explain its role in increasing viral production.

Studies were continued on the effect of chemical agents on FMDV production in cell cultures. Amantadine was tested against FMDV in baby hamster and calf kidney cell cultures. Concentrations of 25 and 250 µg/ml of agent in growth medium containing lactalbumin hydrolyzate and serum were ineffective in reducing virus yields in experiments at a high multiplicity of infection during 5 hours, and in low multiplicity experiments during 18 hours. Amantadine was ineffective in the absence of lactalbumin hydrolyzate and serum in the growth medium in high multiplicity of infection experiments, but did inhibit virus production when infection was at a low multiplicity. This inhibition could be attributed to loosening of cells from the glass surface by the toxicity of the chemical. (ADP a8-17(R))

## G. Studies on Genetic Biochemistry of Foot-and-Mouth Disease Virus

Control of DNA function in cells infected with FMDV. Infection of animal cells with viruses has been shown to shut off normal cellular transcription. It was decided to examine whether this regulation could occur by a histone pathway. The histones of baby hamster kidney cell cultures were examined before and after infection with FMDV, A-119, for changes in heterogeneity, methylation, acetylation and amino acid incorporation. It was found by electrophoretic analysis that infection by FMDV rapidly increased the heterogeneity of cells with arginine rich histones. This suggested an increased histone control mechanism in infected cells. Similar changes were observed by extensive passage of noninfected cells. In support of a histone pathway for decreasing DNA transcription in infected cells, it was



observed that  $^{14}\text{C}$  lysine and  $^{14}\text{C}$  arginine were incorporated at a 50% greater rate into both lysine and arginine rich histones immediately after infection. Likewise, increased regulation by histone after infection was revealed by 3.5-fold decreases in the rates of acetylation (by  $^{14}\text{C}$  sodium acetate) of the N-terminal group of the arginine rich histone and methylation (by  $^{14}\text{C}$  methyl methionine) of the  $\epsilon$ -amino group of the lysine-rich histone.

Reaction of FMDV with Cations and Formaldehyde. Structural changes during the reaction of pure FMDV with cations and formaldehyde were deduced from ultraviolet absorbance measurements. Absorbance-time and absorbance-temperature profiles of FMDV were determined sequentially in the presence of sodium and magnesium ions and in  $\text{CH}_2\text{O}$ . It could be inferred from the profiles that FMDV broke down spontaneously at  $10^\circ\text{C}$  in  $0.001\text{ M Na}^+$  to protein and hypochromic ribonucleic acid. The latter then denatured reversibly when heated.  $\text{Mg}^{++}$  at  $10^{-3}$  markedly suppressed the spontaneous degradation of FMDV; lower concentrations of  $\text{Mg}^{++}$  were progressively less effective.  $\text{CH}_2\text{O}$  at 0.25% appeared to cause the degradation of FMDV at  $10^\circ\text{C}$  at a sodium ion concentration,  $0.02\text{ M}$ , where the virus was otherwise stable up to  $53^\circ\text{C}$ .  $\text{CH}_2\text{O}$  did not lower the degradation temperature as much at higher concentrations of sodium ions where the virus was known to be more stable. Plausible mechanisms were suggested for the action of heat, cations and  $\text{CH}_2\text{O}$  on FMDV. (ADP a8-18(R))

#### H. Effects of Certain Chemical and Physical Environments on Foot-and-Mouth Disease Virus

A  $1\text{ M}$  concentration of hydroxylamine reduced the titer of FMDV O-M11 6-7 logs in 15-30 minutes at temperatures of  $37^\circ\text{C}$ ,  $23^\circ\text{C}$ , and  $4^\circ\text{C}$ . The titer was reduced to the same extent by a  $0.1\text{ M}$  concentration of the chemical at  $4^\circ\text{C}$ , but 28 hours incubation was required. Better immunological preparations, as tested in adult chickens and mice, were obtained when  $0.25\text{ M}$  concentrations were used at  $23^\circ\text{C}$  or  $4^\circ\text{C}$  for 6 to 18 hours incubation.

Argentine strain A-1 FMDV was inactivated with 15 minutes by  $6\text{ }\mu\text{g./ml.}$  concentrations of methylene blue, neutral red and toluidine blue followed by exposure to light rays of incandescent bulbs at a temperature of  $15^\circ\text{C}$ . With crystal violet used similarly, there was only a loss of about 2 logs of virus titer. The methylene blue treated virus was more immunogenic than the others.

A comparison was made between cattle strains of FMDV and their counterparts after adaptation to cell cultures regarding the stabilizing action of divalent cation,  $\text{Mg}^{++}$ , at  $2\text{ M}$  concentration and temperature of  $50^\circ\text{C}$ . The laboratory cattle strain, C-149, was very stable under this treatment and Argentine field strains of types A, O, and C were not as well stabilized. The cell culture adapted viruses from these four strains were entirely unstable with this treatment. This technique may serve as a means of inactivation of cell culture adapted viruses and a means for differentiating such viruses from field or laboratory strains.



At a concentration of 0.05%, acetyleneimine (AEI) inactivated FMDV and allowed retention of immunogenic properties. Similar treatment with beta propiolactone left a residuum of active virus. However, when the latter treatment was preceded by ultraviolet irradiation, inactivation of virus resulted and immunogenic properties were retained comparable to AEI treatment.

Foot-and-mouth disease virus, placed in an antiserum of another type as a simulated contaminant, was inactivated by the addition of 0.3% beta propiolactone. After this treatment, the antiserum retained its neutralizing antibody activity, but there was a three tube loss in complement fixing antibody activity.

The effect of 50, 5 and 0.5 µg. of glutaraldehyde on bovine kidney cell cultures was determined. Some toxic effects, as indicated by rounding of cells, were noted due to 50 µg. of the compound but no toxic effects were produced by 5 or 0.5 µg. of glutaraldehyde. No adverse effects were noted on virus multiplication at these three levels. Glutaraldehyde inactivated approximately 3 log units of FMDV at .05% but only 1 to 0.5 logs of virus at .005% and .0005% after 4 hours at 37°C, respectively. Further studies are necessary to fully evaluate this compound. (ADP a8-19(R))

#### I. Bulk Freeze-Drying of Foot-and-Mouth Disease Virus Vaccines and Antiserums

A study was made of freeze-drying conditions, using cell culture adapted strain A-119 FMDV with various supporting additives as the test agent. The drying conditions that gave the better results were: A residual air pressure of <100 microns, temperature of product at 22 C and condenser at -50 C, a drying time of 36 hours and a product volume of 4.0 ml. Under these conditions, dried virus stored at 4 C in flame-sealed ampules retained full infectivity for one year. The cell culture maintenance fluid was as effective in preserving virus as the additives: skim milk, sucrose, sodium glutamate or normal cattle serum. (ADP a8-20(R))

#### J. Identification, Purification and Chemical and Physical Characterization of Foot-and-Mouth Disease Virus and Other Exotic Animal Viruses

A. Electron Microscopy. African swine fever virus (ASFV) was grown in a stable swine-kidney cell line, and electron micrographs of thin sections of infected cells were made during various stages of viral development. Two hours after infection virus was seen within the cytoplasm. These particles appeared to be those which had been taken up from the inoculum, since no evidence was seen of virus reproduction. In subsequent thin sections taken 24, 48, 72, and 96 hours after inoculation, areas of virus formation appeared and increased in size until the cytoplasm disrupted. A few virus particles were seen in the intercellular spaces after 24 hours, while later micrographs showed many particles budding out through the cell wall. By 96 hours, a large portion of the cytoplasm had been converted to



virus, and the cell disintegrated. The process of virus release was a continuous rather than burst process. During release the particle acquired an outer membrane of cellular material. The structure of fully developed ASFV was unique in processing a very osmophilic hexagonal wall which surrounded an electron lucent region and a central nucleoid. Measurements across the virus and nucleoid ranged from 175-215  $m_u$  and 72-89  $m_u$ , respectively. The period during which ASFV particles were forming in the cell was consistent with the rise of infectivity and hemadsorptinin in cell culture fluids.

FMDV structure. The rotational technique of Markham *et al* was used to compare electron micrographs of highly purified FMDV with rotational photographs of two models most likely to represent the structure of FMDV. The purpose was to determine more accurately the capsomeric structure of FMDV. One model was a 32 subunit rhombic tricontahedron, while the other was a 42 subunit icosahedron. A comparison of the rotational images showed that the 32 and 42 structural unit models were not readily distinguished in most instances. What might be called secondary effects of the reinforcements did indicate, however, that FMDV images were more consistent with the 32-unit structure than they were with the 42-unit structure.

Gamma Irradiation of FMDV. Work has been completed on the gamma irradiation of foot-and-mouth disease virus, type All9, from baby hamster and calf kidney tissue cultures. Comparisons were made on the effect of the cobalt-60 exposure on both crude and pure virus. Virus was maintained at 0°C except during exposure in the  $^{60}\text{Co}$  source where the temperature remained cold to the touch even after a 60 minute exposure. The change in the infectivity of both preparations of virus was monitored as well as the physical state of the pure virus as determined by ultraviolet absorbance-temperature profiles. The approximate intensity of the  $^{60}\text{Co}$  source was 3400 rads/min. at the sample location. Virus infectivity showed appreciable resistance to gamma rays only when protective substances were present. Inactivation rates of crude and pure viruses were approximately 0.4 and 7.0 log units/hr., respectively. Addition of gelatin (0.1%) or cysteine (0.1%) did not stabilize crude virus any further, but caused pure virus to be nearly as stable to gamma rays as crude virus. From knowledge about ionizing effects of gamma rays in aqueous systems, it appears that the additives functioned by neutralizing the newly-formed free radicals and peroxides.

Hydroxymercuribenzoate (HMB) had no effect on either crude or pure FMDV. In contrast to the apparent enhancing effect of cysteine on the infectivity of non-irradiated pure virus, HMB appeared to cause some inactivation, indicating that sulfhydryl groups in the virus may be important to infectivity. From absorbance-temperature profiles on irradiated pure virus, it could be inferred that considerable degradation of virus occurred within 1 minute of exposure. At 10 minutes, it could be deduced that scission of the sugar-phosphate backbone structure of viral RNA had commenced. At 30



minutes, backbone scission was nearly complete, and destruction of purine and pyrimidine rings had commenced. At 60 minutes, both kinds of breakage appeared to be complete.

Amino Acid Composition of FMDV. Analysis has been made of the amino acid composition of FMDV, types A<sub>119</sub>, O<sub>9</sub>, and C<sub>3</sub> produced in baby hamster kidney cultures and purified by procedures developed previously. Analysis was also made of type A<sub>119</sub> virus from cattle which had been passaged only a few times in calf kidney tissue cultures. Statistical comparisons revealed no differences at the 0.05 level of significance in the amino acid content of type A<sub>119</sub> virus whether grown in baby hamster or calf cells. However, type O<sub>9</sub> virus differed from type A<sub>119</sub> in its content of alanine, leucine, tyrosine and possibly histidine. Type C<sub>3</sub> differed in its content of threonine, serine, alanine, valine, isoleucine, tyrosine, phenylalanine, lysine and possibly tryptophan. Type O<sub>9</sub> and C<sub>3</sub> differed from one another in their content of all the amino acids listed previously, as well as in glycine and arginine and possibly 1/2 cystine.

Application of Digital Computers to Ultracentrifugation. An investigation of the applicability of digital computers to analytical ultracentrifugation has been completed. Since Plum Island does not possess a computer, the facilities of other institutes both in the United States and England, were employed. Three major problems were examined which apply to virus research: a) determination of sedimentation coefficients from data of radial position of the solute as a function of time; b) determination of the molecular weight from solute redistribution data, and c) determination of interaction constants from summary data of sedimentation coefficients as a function of concentration. It was concluded that a) ultracentrifuge calculations which are too tedious to compute manually can be computed automatically; b) digital computers permit assessment of internal errors and the building in of safeguards; c) both linear and non-linear least squares statistics can be used, and d) certain specified criteria should be applied when using computer programs developed by others. (ADP a8-25)

#### K. Immuno-Chemical Investigations of Foot-and-Mouth Disease

Cattle infected with foot-and-mouth disease virus produce four, and possibly five different molecular species of antibodies. The antibody detected first is a 19S y<sub>1</sub> -globulin that reaches a high level by the 7th day but is not readily detectable after about 30 days. By the 14th day, antibodies are present that have lower sedimentation rates. These have been fractionated into three or four different electrophoretic classes of antibodies. One of these antibodies is the 7S y<sub>2</sub> type and it did not fix complement as well as the faster migrating antibodies. The persistence of the different antibody types is apparently dependent upon the method of exposure to virus antigen.

Conditions for testing bovine and swine serum by the complement-fixation technique were established. (ADP a8-26)



#### L. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products

A study of the survival of FMDV in cattle hides was prompted by the great numbers of hides that are annually imported from FMD countries. Experimentally infected cattle were slaughtered at various times after inoculation and hide samples were taken from shoulder, lumbar, inner thigh and perineal regions. The samples were shaved and aseptic precautions were taken. With all seven types of FMD, virus was regularly detected in fresh hide samples taken from 32 cattle during the period of viremia. From 6 to 28 days after inoculation, virus was found in hides of 14 of 22 cattle, with the longest persistence of 18 days. The highest virus titer found in hides was  $10^{5.5}$  cell culture plaque-forming units per gram (PFU). There was no significant difference in titers of virus from the various skin areas. A study of FMDV survival in dried, salted and chemically treated hides is in progress.

The importation of various glands and tissues from FMD countries for production of biological products prompted a study of the survival of virus in the central nervous system structures. Foot-and-mouth disease virus was detected in high titers in the pituitary gland of experimentally infected cattle from the early clinical to the early convalescent stages of the disease. Virus persisted for as long as two days after viremia ended. The highest titer obtained was  $10^{6.8}$  cell culture PFU/gm. and the virus titers in the pituitary were equal to or higher than those found in the blood.

Virus was also isolated from the spinal cord, pineal body, cerebrum and cerebrospinal fluid, but less frequently and, with lower titers than from the pituitary. Virus was not isolated from cerebellum, medulla or hippocampus. Additional studies are in progress on survival of virus in other endocrine glands. (ADP a8-28)

#### M. Studies on the Biological Mechanisms of Natural Resistance and Susceptibility of Foot-and-Mouth Disease Virus

Mice from litters consisting of 5 and 10 animals and therefore of different weights although of the same age, were equally susceptible to FMDV at 7, 14, and 21 days of age. Their response at 21 days of age indicated that some litters of mice were more susceptible than others. Extension of this work to 28- and 35-day-old mice revealed that the mice of certain litters were still susceptible while those from other litters were resistant to the virus inoculum used.

Efforts were made to learn if a relation existed between in vitro virus production by mouse kidney cells and the in vivo response of the cell donor. Single kidneys were surgically removed from adult mice, and virus production was determined in suspensions prepared from the kidneys. The recovered mice were inoculated with virus and their response was observed. A meaningful correlation between the amount of virus produced in vitro and the animal's response was not obtained, due at least in part to the fact that



the mice were more susceptible following the operation. Similarly, comparison of in vitro virus production with the response of litter mates of the cell donors also failed to show a consistent pattern perhaps due to the variation in response of 35-day-old mice.

Inoculation of suspensions of minced kidneys from 7- and 35-day-old mice resulted in adsorption of 80-90% of the virus over a 3 to 4 hour period regardless of the virus concentration at the start. To date, the experiments indicate that 10-20% of this virus population is a variant which is not adsorbed by these cells under the experimental conditions.

In the performance of the plaque assay, incubation of cultures with confluent cell sheets at 37, 30, or 24°C before inoculation is satisfactory if a 37°C temperature is provided during the adsorption and subsequent incubation period. Rotation after 30 minutes of adsorption is unnecessary. A 2% serum concentration in the overlay medium is as satisfactory as 10% for plaque formation. (ADP a8-29)

#### N. Biological Alterations of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures

Foot-and-mouth disease virus type A, strain 119 that had undergone primary modification by chronic residence in primary calf kidney cell cultures had no practical value as an immunizing agent. Subclinical infection required to produce immunity in cattle occurred regularly only when small carefully regulated doses were given. Larger doses sometimes failed to produce subclinical infection on account of an interference factor in the virus, or produced mild clinical signs of the disease.

Significant improvement was obtained by secondary modification of the virus effected by a single passage in cattle. The secondary modified viruses were obtained from the blood of cattle with subclinical infection from the primary modified virus. One secondary modified virus consistently produced subclinical infection (viremia without clinical signs of the disease) in a wide range of dosage. Cattle inoculated with this virus resisted challenge with virulent foot-and-mouth disease virus 22 days later.

Lyophilization damaged the secondary modified virus to the extent that some inoculated animals developed signs of the disease and some failed to become immune.

Doses of these modified viruses that produced subclinical infection in cattle, produced clinical infection in swine, although the disease was mild as compared with natural infection. (ADP a8-30)



#### O. Morphologic Aspects of Virus-Cell Relationships

Growth rates of cell "lines" developed from cell cultures surviving infection with FMDV were apparently less than those of conventional type cell lines. One such "line" was obtained from primary swine kidney cultures and one from primary culture of a canine seminoma. Their status (primary or permanent) remains to be determined.

Three types of unmodified FMDV (A-119, C3 CANEFA and O-M11) were propagated in primary canine kidney cell cultures. Low titer virus yields indicated intermediate degree of cell susceptibility to the viruses. Higher titers of canine cell passaged viruses were obtained by subsequent passage in primary lamb testis cell cultures. (ADP a8-31)

#### P. Diagnostic and Immunizing Procedures for Contagious Bovine Pleuropneumonia

The value of mice and cell cultures for propagation of the etiologic agent has been investigated. In both systems, Mycoplasma mycoides will reproduce and persist but neutralization of the activity has not been successful. M. mycoides appears to be extremely sensitive to ethylene oxide gas as the growth of cultures were inhibited in media previously exposed to the gas.

A variety of diagnostic and reference materials have been produced for assistance in diagnosis of contagious bovine pleuropneumonia. These materials consist of - rabbit-immune serum, diagnostic antigens, and experimental serums produced in guinea pigs.

Work has also been conducted to develop a fraction of the organism which, when inoculated into cattle immune to contagious bovine pleuropneumonia, will cause a delayed skin reaction. The development of such a method would assist, immeasurably, in arriving at a diagnosis. (ADP a8-32)

#### Q. Studies on Foot-and-Mouth Disease Virus (PL 480 project)

Under a PL 480 Grant, research is being conducted on foot-and-mouth disease virus (FMDV) at the Instituto Biologica, Sao Paulo, Brazil. During a 6-months period 27 samples were collected from spontaneous cases of foot-and-mouth disease. Thirteen were positive in direct complement fixation tests. Types isolated were A, O, and C. Investigations on comparative observation on bovine and swine kidney cell cultures revealed that swine primary cultures are always highly susceptible, and the bovine cultures usually presented variable response to the virus infection. Doses which induced an extensive cytopathic effect in "normal" primary bovine cultures, produced only foci of dead cells in the resistant ones. Studies on 5 long-term bovine cultures showed that they had lost most of their primitive susceptibility to FMDV, and they had a diploid number of chromosomes and a low growth rate. Long term observations on 3 swine celllines showed they had maintained their susceptibility to the same virus. (E3-ADP-2)



R. Studies on Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of FMD in Turkey  
(PL 480 project)

Under a PL 480 Grant to the Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey, research was conducted on various types of foot-and-mouth disease virus indigenous to Turkey, and on the production of a vaccine for the control of the disease in Turkey.

SAT 1 Ova. F4-Kn9 strain virus was obtained from sheep. This SAT 1 type of foot-and-mouth disease infection was widespread in Turkey. It was easy to adapt to sheep. Work on other strains of the virus was stopped for the present. Ubetinis' method was found to be satisfactory for trypsinization of kidney tissue cells. Serums from horses, healthy unvaccinated cattle and recovered cattle were found to be satisfactory for use in calf and lamb kidney cell cultures. The entry of Type A FMD virus from Iran resulted in a delay of the work on SAT 1 until new facilities could be established. The cell culturing studies are progressing satisfactorily. (A22-ADP-8)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Immunological Investigations

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PARASITES AND PARASITIC DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 19.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Gastro-Intestinal Nematodes of Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet 1.0 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus 1.0  
at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle,  
Including Anthelmintic Treatment 1.5 at the University of California,  
Davis, under a cooperative agreement with the ARS-USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and  
Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah,  
and under a cooperative agreement with the Utah Agricultural Experiment  
Station, Logan, Utah.

Host-Parasite Relationship of Intestinal worms, Cooperia spp. in Cattle 1.0  
at the Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites  
of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory,  
Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the  
Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative  
agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory,  
Beltsville, Maryland, and through a Memorandum of Understanding and other  
agreements in cooperation with the State Experiment Stations in California,  
Illinois, Louisiana, Nevada, and State Veterinarian of Tennessee, the USDA  
Entomology Research Station, Kerrville, Texas, and the Delta Branch  
Experiment Station, Stoneville, Mississippi.

Interrelationships of Diet and Parasitic Infection in the Production of  
Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the  
Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with emphasis on Stephanofilarial Species 1.0 at the  
Animal Disease and Parasite Research Division Regional Animal Disease  
Laboratory, University Park, New Mexico.

Effect of Stocking Rate and Rotational Grazing on Internal Parasitism of  
Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Environmental Factors Influencing Parasites and Parasitic Diseases of  
Economical Importance in Ruminants (Cattle, Sheep, and Alpacas) (PL-480 Peru)

Investigations on Anaplasmosis, Piroplasmosis and Babesiallosis of Cattle  
are under way through a PL 480 Grant at the School of Veterinary,  
Montevideo, Uruguay (PL 480 Uruguay)



## PROGRAM OF STATE EXPERIMENT STATIONS

The State Stations have a long term program covering basic and applied aspects of the major internal parasite problems of cattle. Twelve states in the Western Region and the Department are cooperating in regional research on cattle nematode problems (W-35). Informal coordination is maintained with States in the Southern Region also working in this subject matter area.

Basic research is in progress to establish how nematodes damage the host animal, interfere with nutrition and result in disease. Research on the biochemical systems concerned with parasite metabolism and the effect of anthelmintics on these systems is providing basic information necessary in developing improved therapeutic controls. Other studies are centered on means for reducing the exposure of cattle to infective stages of parasites. Systems of grazing management and feeding procedures are being evaluated and factors which favor over-wintering survival of parasite larvae are being determined. The relationship between types of pasture forage and the degree of parasitism are being determined and the micro-climatic conditions conducive to parasite larval infectivity are being established. Immune mechanisms involved in resistance to parasites are being determined.

A number of states are engaged in studies on the nature of Anaplasma, the life cycle of this parasite, methods for immunization against anaplasmosis and procedures for eradicating the disease. Other work is in progress on the life cycle and means of controlling coccidiosis of cattle.

The total State scientific effort devoted to this research is 29.1 professional man-years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Etiological Factors Influencing Gastro-Intestinal Nematodes of Cattle

The report on research conducted at Experiment, Georgia, under the auspices of the Regional Animal Disease Laboratory at Auburn, Alabama, shows the use of vermiculite as a culture medium for larvae of nematodes of ruminants was investigated. Feces containing eggs of Oesophagostomum radiatum, Cooperia pectinata, C. oncophora, Trichostrongylus colubriformis or T. axei was collected from monospecifically infected calves and cultured in either the conventional sphagnum moss or vermiculite (Terra-Lite Brand, Zonolite Company). Average larval recovery was higher from cultures made with vermiculite than those made with moss, although the differences were not significant. Larvae in sheep pellets were also cultured successfully with vermiculite. In general, vermiculite was more advantageous for routine culturing, being cleaner, more hydrophilic, more economical, and conveniently packaged. Furthermore, the granular nature of vermiculite makes it easier to mix and spread out on the water surface of a Baermann funnel.

(Alabama and Georgia) (ADP 61-6(R))

## B. Host-Parasite Relationship of Coccidial Parasites of Cattle.

Scientists at the Animal Disease and Parasite Research Division's Regional Animal Disease Laboratory at Auburn, Alabama, reported the following: When 20% antiformin and 1.0 M sulfuric acid were used consecutively or separately there was very little reduction in sporulation or destruction of oocysts of Eimeria ellipsoidalis, E. auburnensis, and E. Bovis, but when the two solutions were combined, the mixture nearly completely prevented sporulation and destroyed almost all of the oocysts and contents. Half-strength solutions were not as effective, as there was no evidence of destruction and all oocysts were not prevented from sporulating. Half-and quarter-strength solutions were more effective in reduction of sporulation of oocysts of E. auburnensis than oocysts of E. bovis.

Additional data were obtained on the life cycle of Eimeria cylindrica in calves. Six young calves were inoculated with 50,000 to 240,000 oocysts of E. cylindrica to determine the prepatent period. Five of the six became infected and had oocyst outputs ranging from light to heavy. The prepatent period was 9 days postinoculation (PI) in one, and 10 days in the other four. Peak days of oocyst discharge were 12, 12, 19, 13, and 10 days PI. Patent periods lasted 8, 5, 21, 13, and 12 days. Data were obtained on the endogenous stages of the parasite in three other calves, two killed at 8 days PI and one killed at 11 days. In the first calf killed at 8 days, macrogametocytes were found in the crypts of Lieberkuhn in the lower small intestine. In the other calf killed at 8 days PI, immature macrogametocytes measuring up to 19.5  $\mu$  were found in the last portion of the small intestine in the glandular crypts.

Two calves inoculated with oocysts of Eimeria canadensis were killed at 9 and 16 days PI to obtain information on the endogenous stages of the life cycle. At 9 days PI, immature schizonts were found in the small intestine in the apices of villi one foot below the stomach and at 24 ft. above the ileocecal valve. At 16 days PI, merozoites ranging up to 18.2  $\mu$  were found in great numbers from C plus 42 ft. to C plus 6 feet. Schizonts were most numerous at C plus 12 to C plus 24. They were macroscopic and were located in the lacteals of the villi, resembling those of E. bovis and E. auburnensis. Microgametocytes resembled those of E. auburnensis; the microgametes were arranged peripherally and invaginated at places.

(Auburn, Alabama)

(ADP bl-23(Rev.))

## C. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle including Anthelmintic Treatment.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reports on their investigations as follows:



Anthelmintic Studies (a) Disophenol (DNP), an excellent anthelmintic against a narrow spectrum of nematodes, was found to have significant action against Fasciola hepatica, the common liver fluke of sheep and cattle. (b) Haloxon at 50 mg/kg in both sheep and cattle was found to remove greater than 90% of the more important species of gastrointestinal parasites in California. (c) Field trials with Thibenzole indicated that a dosage of 3 grams/100 lbs. was equal to that of 5 grams/100 lbs. in cattle as a prophylactic treatment.

Physiological Studies (a) Water balance studies indicated a marked (50%) reduction in water intake and output of cattle suffering from gastrointestinal parasitism. It was further noted that a much greater reduction (dehydration) of the extracellular water compartment occurs than is indicated by the plasma volume.

Development of an experimental model for laboratory study of physiological alterations. Data so far collected indicate that Obeliscoides cuniculi infection in the rabbit may be valuable as a model for studying many of the alterations which occur in cattle as a result of gastrointestinal parasitism. (California) (ADP bl-25)

#### D. Investigations on Trichomonad Parasites.

Studies conducted at the Division's Regional Animal Disease Laboratory at Logan, Utah, included the production of antisera in rabbits to six strains of Trichomonas foetus. Antisera was produced in fiscal year 1964 to two strains of T. foetus, and in 1965 to four additional strains which were acquired from widely divergent sources in this country and in Europe. The antisera were produced by two graded series of five intravenous inoculations of live, washed organisms given twice weekly with a three-week interval between the two series.

Quantity of agglutinating antibody was determined by exposing live, washed trichomonads to various dilutions of the antiserum in Ringers solution. Each trichomonad was tested against its homologous antiserum and against each of the other five antisera, and each antiserum was tested against its homologous trichomonad and each of the other trichomonads. Homologous titers were 20480, 20480, 5120, 5120, 2560, and 1280 which, with the exception of the 1280 titer, indicates good strong reactions. Heterologous titers ranged from 160 to 20480.

Preliminary analysis of the agglutination results indicates there are antigenic differences in the various strains of T. foetus.

Gel diffusion studies were made comparing the six strains by the Ouchterlony gel diffusion technique. The suspended particulate material in the antigen varied in size, and some of the crude preparations plugged the gel preventing migration of antigens and subsequent formation of precipitin lines.

After centrifugation of the antigen at 37,000 gravities, an antigen of considerably better quality was produced. With the improved crude antigen, precipitin lines formed indicating the presence of four to seven antigen-antibody systems. Use of the improved antigen has shown a varying number of precipitin lines form by reaction of the medium ingredients and bovine serum which is in the medium with the antiserum.

Tests made on bulls from a locality in Utah which has been troubled by trichomoniasis for the past seven years and with which we have worked closely, revealed no trichomoniasis at the present time. (Logan, Utah) (ADP bl-26)

E. Host-Parasite Relationship of Intestinal Worms, Cooperia species, in Cattle.

Reported research from the Division's Regional Animal Disease Laboratory, Auburn, Alabama, showed that cattle may be immunized against pathogenic worm parasites by controlled inoculations with closely related species. Three species of Cooperia--C. punctata, C. pectinata, and C. oncophora--are common parasites of cattle. The latter species is relatively non-pathogenic to calves, whereas the other two species are equally harmful. Calves inoculated with C. oncophora have become almost completely immune to challenge inoculation with C. pectinata and partially immune to C. punctata. Individual animals, however, failed to become immune to subsequent challenge with the original species and these calves were also unable to resist challenge with the related species.

(Auburn, Alabama) (ADP bl-27)

F. Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle.

Scientists at the Beltsville Parasitological Laboratory reported that malnutrition, experimentally produced in calves from 12 to 17 weeks of age, prior to infection with gastrointestinal nematodes and resultant stunting, influenced the level of parasitism acquired by them while grazing pastures, as indicated by higher worm-egg counts than those of similarly exposed full-fed calves. The driest summer on record complicated the interpretation of subsequent postmortem data insofar as determining the effects of malnutrition on the development of nematode parasitism. However, it presented an unusual opportunity to study the epidemiology of the parasitism in question under drought conditions.

Heavy contamination of the pastures with manure containing large numbers of nematode eggs during the drought led to the development of very heavy concentrations of infective larvae on the forage during the cooler weather of early fall which followed a short period of normal rainfall. The continued grazing of these heavily contaminated areas during the mild but moderately dry conditions of late fall led to extremely heavy infection of the cattle with an average of 249,000 worms per animal. The predominant



species was Ostertagia ostertagi, the medium stomach worm. This finding indicated that its free-living stages were able to survive conditions that were lethal to those of other species.

The ability of eggs of the beef tapeworm (Taenia saginata of man) to cause formation of cysts (Cysticercus bovis) in the muscles of cattle apparently was reduced rather markedly by exposure to 50,000 r, 100,000 r and 200,000 r of x-rays and in proportion to the amount of exposure. An animal that had been vaccinated with eggs exposed to 200,000 r, and was given a large dose of normal eggs subsequently, was found to be free of cysts. An average of about 3,300 cysts developed in two unvaccinated controls that received an identical dose of normal eggs. (Beltsville, Maryland) (ADP bl-28)

#### G. Etiology and Immune Response of Cattle to Winter Coccidiosis.

Reports on research conducted at the Division's Regional Laboratory at Logan, Utah, show that six experiments were conducted which involved coccidial infections with Eimeria bovis or Eimeria zurnii in Holstein-Friesian calves. One experiment dealt with the effect of prolonged low-level inoculations with sporulated oocysts of Eimeria bovis on the development of immunity in calves. Ten, 100, or 15,000 oocysts/day were given calves for 60 days. Clinical signs were exhibited only in calves receiving 15,000/day. The highest oocyst discharge also occurred in this group, but the number of days oocysts were discharged was about the same in all groups. All groups of calves exhibited resistance to reinfection when challenged with 500,000 oocysts at the end of 60 days inoculation.

Three experiments involving calves inoculated with Eimeria zurnii were completed. All were designed to gain information on methods of producing reliable experimental infections. Cortisone injections were used unsuccessfully in an attempt to suppress the immunogenic mechanism in the calf and allow the coccidia to invade the host. The sporulated oocysts were treated in such a way that the exterior wall would be dissolved, thus making the oocyst more susceptible to digestive fluids, including enzymes. This technique was also unsuccessful.

In the second experiment both sporulated and unsporulated oocysts were irradiated at 10,000 r, 50,000 r, 75,000 r, or 100,000 r in a cobalt-60 source. In the first part of this experiment inoculation of calves with sporulated oocysts produced results similar to those reported last year and to those reported above. However, the results were more conclusive. A challenge inoculation after recovery from the first inoculation indicated that immunity against reinfection was present in the calves which had previously exhibited clinical signs and discharged oocysts, i.e., calves receiving oocysts irradiated at 10,000 r. Little or no resistance was observed in the other 3 groups of calves. These results indicate that irradiated oocysts have no special value as immunological agents against coccidiosis caused by Eimeria bovis. (Logan, Utah)



Studies in cooperation with the Utah State University at Logan are reported as follows:

Description of the sporulated oocysts and sporozoites of four species of bovine coccidia. The sporulated oocyst is the stage of the coccidial life cycle most favorable for identification as to species. The bovine coccidia have been described only incompletely with respect to this stage. The description, including drawings, have been completed of the sporulated oocysts and free sporozoites of the four species most common in the Logan area, namely, E. bovis, E. zurnii, E. ellipsoidalis, and E. auburnensis. The free sporozoites were obtained for observation by causing the oocysts to excyst in vitro. The sporozoites of E. zurnii were found to have only one relatively small refractile granule in each sporozoite instead of two relatively large refractile granules as in each of the other three species. This morphological difference may be associated with the peculiar epidemiological pattern exhibited by E. zurnii, for example, its association with "winter coccidiosis" and the difficulty of inducing experimental infections with this species.

Cytological study of coccidia. A cytological study of the stages of Eimeria bovis and other bovine species of coccidia was undertaken to obtain fundamental information useful in developing methods of prevention and control of coccidia. It was necessary to section oocysts because of the impermeability of their wall. Work has demonstrated its feasibility.

First-generation merozoites of Eimeria bovis were obtained in large numbers from mature schizonts, after concentration of these by repeated washing and sedimentation. The appearance of living merozoites, as well as their flexing and gliding movements, were described with the use of the phase-contrast microscope. In specimens stained with protargol the anterior portion of the body was found to have a cap-like covering with a terminal pore, and a median rod-like structure. Prominent granules occurred in the posterior 2/3 of the body, with one granule characteristically located at the posterior extremity. The nucleus was in the posterior 1/3 of the body. In Feulgen and acridine orange preparations the chromatin was arranged as a ring at the periphery of the nucleus; at irregular intervals there were coarse clumps, usually 3 to 5 in number. Numerous small glycogen granules were present in the posterior 2/3 of the body. No sudanophilic lipids were demonstrated. The entire body of each merozoite showed a diffuse positive reaction with the ninhydrin method. These merozoites were found to be similar in certain morphological features to Toxoplasma gondii.

Nitrofurazone as a prophylactic agent against experimental bovine coccidiosis.

In 3 experiments, each with 12 calves about 2 months old, the administration of nitrofurazone in the feed at 5.0 or 7.5 mg/kg of body weight daily for 6 weeks, beginning 4 days before inoculation of 50,000 to 100,000 Eimeria bovis oocysts, did not prevent the occurrence of coccidiosis. In each of these experiments the calves were allotted to 3 groups, each including 2



inoculated calves housed in the same pen with 2 uninoculated calves. Little or no infection was observed in the uninoculated calves. In 2 of the 3 experiments, sporulation of oocysts apparently did not occur in the pens during the course of the experiments because of low temperatures; in the 3rd experiment the calves evidently had some degree of immunity as a result of natural infections. The weight gains of the treated calves were not consistently different from those of the untreated calves, but the calves given 7.5 mg/kg made smaller average weight gains than those given 5.0 mg/kg. The results of 1 experiment with 8 calves, 2 months old, indicated that nitrofurazone in the feed at 10 mg/kg for 3 weeks beginning 1 week before inoculation of 100,000 oocysts each of E. bovis and Eimeria zurnii, had only questionable value in preventing coccidiosis. In 1 experiment with 6 calves about 4 months old, nitrofurazone administered in gelatin capsules at 30.0 mg/kg for 4 days starting 15 days after inoculation of 100,000 E. bovis oocysts, was effective in controlling coccidiosis.

Amprolium for control of coccidiosis in calves. Sixty-nine calves were used in 6 experiments to determine the efficacy of amprolium in controlling Eimeria bovis infections. In one of these experiments, 3 additional calves were treated with ethopabate. In each experiment three or four groups each of 3 calves about 2 weeks old, were inoculated with 50,000 or 100,000 oocysts; two or three of these groups were given liquid amprolium in the milk. In each of three experiments, one group of 3 calves was left uninoculated until 30 days after the original inoculation, then all groups were challenged with 1 million oocysts. In five experiments, treatment at 16.25 mg/lb for 21 days, beginning on the day of inoculation, provided good to excellent control of coccidiosis, as did such treatment at 65 mg/lb and 10 mg/lb in two experiments.

In four experiments, calves treated at 65 mg/lb for 5 days beginning 13 days after inoculation, had less severe signs of coccidiosis and discharged fewer oocysts than untreated calves, but the results of this treatment were not as good as those of the 21-day treatments. In one experiment, treatment with amprolium at 65 mg/lb or ethopabate at 1 gram/lb for one day, 13 days after inoculation, had little or no effect on the infections. Calves which had been treated had less severe infections after challenge than did controls not previously inoculated. (Logan, Utah) (ADP 61-29)

#### H. Investigations of Anaplasmosis

Research at the Beltsville Parasitological Laboratory has shown that a partially purified antigenic protein has been isolated from red blood cell hemolysates obtained from cattle with acute anaplasmosis. Concentration, characterization and immunogenic studies on the material are under way.

Thin-section studies on the ultra-structure of A. marginale have revealed that the parasite contains, in the bovine red blood cell, from one, to at least six, smaller organized units or bodies. The detailed structure of these sub-units has not been clearly demonstrated. A mild type of

A. marginale, naturally occurring in the United States, has been observed to be of similar pathogenicity to A. centrale, the so-called vaccine type used in Africa and elsewhere for premunition.

Field tests are in progress to determine if a "dead Anaplasma" antigen will protect cattle against natural exposure in a degree sufficient to prevent economic losses from the disease. (Beltsville, Md.) (ADP bl-30)

#### I. Histochemistry of Gastro-Intestinal Nematodes of Cattle

Research work at the Division's Regional Animal Disease Laboratory at Auburn, Alabama, was reported as follows:

Studies conducted on the histochemistry of the host response to the presence of larval nodular worms, Oesophagostomum radiatum, in the wall of the small intestine of the calf have demonstrated that the presence of these larvae is associated with a decrease in the connective tissue protein collagen and an increase, real or apparent, in glycoprotein around the site of the larval worms. These alterations in the chemistry of the tissue of the calf appear early in the infection and disappear as healing is completed.

The presence of the larval worms is also associated with an increase in the activity or amount of the enzymes alkaline phosphatase, acid phosphatase, and non-specific esterase in the vicinity of the lesion. The increase in these enzymes is apparently associated with metabolic changes in the cells of the host as they respond to the presence of the parasites. Infection by the young nodular worms produced no effect on the distribution and abundance of the enzyme leucine aminopeptidase during the stages of the infection studied.

Ketoenolic lipoids, originally discovered in certain other animals by the Japanese scientist Yukio Hamazaki, were found for the first time in the tissue of cattle and nematodes. The amount of these chemicals is increased around young nodular worms in the wall of the small intestine during some, but not all stages of nodular worm disease in cattle. These same chemicals also occur in the intestines of young nodular worms.

(Auburn, Alabama) (ADP bl-32)

#### J. Parasites of Cattle with Emphasis on Stephanofilarial Species

Studies made at the Division's Regional Animal Disease Laboratory at University Park, New Mexico, have shown that Stephanofilaria stilesi is a worm parasite which causes extensive sores on the skin of cattle. This chronic condition is called "stephanofilarial dermatitis." It is widespread in the United States and other parts of the world and is of much economic importance. Research based on natural and experimental infections at the University Park Field Station proved that the disease is transmitted by horn flies. Effective medicinal treatment directed at the worms in the



lesions is not yet available. Perhaps diligent control of horn flies would result in a lower incidence of the disease.

(University Park, New Mexico) (ADP bl-33)

K. Effect of Stocking Rate and Rotational Grazing on Internal Parasitism in Beef Cattle

This work was done at Experiment, Georgia, under the auspices of the Division's Regional Animal Disease Laboratory at Auburn, Alabama. The report shows that experiments suggest that rotational grazing results in increased rate of stocking of the pastures and consequently increases parasitism in beef cattle. Two lots of winter temporary pasture were stocked at the same time - one was grazed continuously and the other was grazed on a four-way rotational system. A third lot was also rotationally grazed, but the stocking rate varied with the carrying capacity of the pastures. The steers from the two rotationally grazed groups had more worms and made a lower average daily gain. The steers grazed rotationally where the stocking rate varied had the greatest number of worms. Although various factors may be responsible for an increase in parasitism, the increased stocking rate is very likely the most significant factor.

(Experiment, Georgia) (ADP bl-34)

L. Under a PL 480 grant to the School of Veterinary Medicine, University of San Marcos, Lima, Peru, research is being conducted on Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (cattle, sheep, alpacas). This project was reviewed during 1964 by a Department scientist who visited the Principal Investigator in Peru. The reviewer explained the use of several methods adaptable under conditions in Peru for improvement of the investigations.

The accomplishments for the third year of research are 1) collection of climatic data in relation with the seasonal incidence of parasitic diseases of livestock; 2) preparation of a calendar of treatment and control of parasitic diseases of livestock, according with the management and seasonal occurrences of these diseases; 3) a check list of parasites identified in our laboratory of parasitological research, during the period 1961 to 1964; 4) bionomic of fresh water snails, transmissors of Fasciola hepatica, and 5) a pamphlet on Parasites and Parasitic Diseases of Lamb pacos (Alpacas) in Peru.

(PL 480) (S8-ADP-1)

Diagnosis and Methods of Prevention and Treatment of Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle and Further Characterization of the Causative Agents. These investigations were conducted under a PL 480 grant to the School of Veterinary Parasitic Diseases, Montevideo, Uruguay. The work involved studies on 1) Tick development in vitro - Results - Nymph stage was achieved. Nymphs were kept alive for a period of 45-50 days in vitro. 2) Tissue cultures from bovine infected with Babesia bigemina - Results - Dermal tissue, spleen and brain were studied. 3) Tissue culture from bovine infected with Anaplasma marginale and uninfected bovine -

Results - Primary cultures showed several macrophages with parasited red cells. 4) Electrophoretic studies of bovine serum - Results - In the group inoculated with Anaplasma centrale serum protein variations occurred. The variations in general appeared in irregular form from one animal to another, differently to what occurs in anaplasmosis by A. marginale. 5) Electrophoretic studies on bovine serum - Results - Serum protein of cows infected with A. centrale and challenged with A. marginale were studied in 22 animals: a) pre-patent period - during this period only a decrease of alpha-globulin value was observed, b) patent period - at the beginning of this period, value of beta-globulin increased - remained until incidence of A. marginale, then returned to pre-inoculation value, gamma-globulin and total proteins decreased, and alpha-globulin in this period returned to pre-inoculation value. c) when red blood cells decreased under one percent; total serum protein and gamma increased, while albumin alpha and beta-globulin remained at pre-inoculation levels. d) no significant variations occurred at serum protein levels in the control cows free from A. centrale and A. marginale.  
(PL 480 Uruguay) (S9-ADP-1)

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**DAIRY CATTLE INSECTS**  
**Entomology Research Division, ARS**

Problem. Flies, mosquitoes, grubs, lice, and ticks are common pests of dairy cattle that cause important losses in all parts of the United States. Heavy attacks by biting flies lower milk production by 5 to 20%. Total losses to dairy cattle attributable to insects and ticks are estimated to exceed \$200 million annually. Certain insect pests are also involved in the transmission of diseases of dairy cattle. Methods of control for dairy insects have received setbacks during recent years because the best available insecticides and most promising new materials produce residues in milk. In addition, house flies around dairy establishments have developed resistance to DDT and other insecticides. There is, therefore, great need to find safe, effective, non-residue insecticides and repellents to control these insects and ticks. Effective systemic insecticides and ways of administration which would avoid residues are needed to combat grubs in dairy cattle and to prevent the face fly and horn fly from breeding in the manure. New approaches to control, including radiation and chemosterilants, need to be explored further to determine their feasibility for the control of several dairy-cattle pests. Research should be continued to support the Southwestern screw-worm eradication campaign. Efforts also should be made to find and evaluate insect pathogens, parasites, and predators for controlling certain dairy-cattle pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycle to serve as a basis for the development of more effective and safer methods of control. Research is also urgently needed on the role of insects in the spread of diseases of dairy cattle.

**USDA AND COOPERATIVE PROGRAM**

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of dairy cattle. Studies are conducted on the biology, physiology, genetics and nutrition of the screw-worm fly, stable fly, horn fly, horse and deer flies, the face fly, mosquitoes, and other pests; on the nature of insect resistance to insecticides; on the mode of action of insecticides and on their absorption, metabolism and excretion by insects; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is directed towards the development of more effective conventional and systemic insecticides and protective treatments for the control of dairy-cattle pests. Studies are conducted to determine the occurrence of insecticide residues in the tissues and the milk of treated animals. Minor attention is given to the development of sanitation and management procedures and to biological control, especially parasites and predators, for controlling the face fly, stable fly, horse fly, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to develop physical and mechanical methods of control, to evaluate traps and devices for estimating



and controlling natural insect populations and improved or special equipment for the application of insecticides to dairy cattle. Limited research is conducted on the role of insects and ticks as vectors of animal diseases, with special emphasis on bovine anaplasmosis. The research is conducted in major laboratories at Kerrville, Tex., Corvallis, Oreg., and Gainesville, Fla., and at satellite stations at Beltsville, Md., Stoneville, Miss., Lincoln, Nebr., and Fresno, Calif. Research is conducted at McNeese State College, the University of Southwestern Louisiana, and the University of California under contracts.

The Federal scientific effort devoted to research in this area totals 16.6 professional man-years. Of this number 6.4 is devoted to basic biology, physiology and nutrition; 3.7 to insecticidal and sanitation control; 2.5 to insecticide residue determinations; 0.3 to biological control; 1.9 to insect sterility, attractants and other new approaches to control; 0.3 to evaluation of equipment for insect detection and control; 0.7 to insect vectors of diseases; and 0.8 to program leadership. The Federal support devoted to research in this area under contracts totals 0.8 man-years, of this number 0.2 is devoted to basic biology, physiology, and nutrition, 0.3 to insecticide and sanitation control and 0.3 to biological control.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Valuable information on insects affecting dairy cattle is being provided by research in the States. Studies are in progress to determine the abundance, geographical distribution, seasonal variations and economic importance of pest species. Rearing methods are being developed to provide insect specimens (1) for laboratory studies involving the effects of ecological factors on growth and survival; (2) for studying the microorganisms normally present in pest insects; (3) for cattle disease transmission tests performed to determine which insects may serve as vectors; and (4) for control studies.

Various substances are being evaluated for their attractant or repellent effects on such pest insects as flies. Those attractants which exert a significant effect are incorporated as baits with new insecticides or chemosterilants. Various other application methods are also being evaluated.

The development of resistance to insecticides in flies has brought about research to determine methods of combatting it as well as initiating a search for new chemicals. Studies are in progress to determine the effects of repeated heavy insecticide dosages as opposed to light doses, and the influence of fly behavior, development and reproductive capacity on resistance. The mechanism of resistance in the insect in relation to penetration of the integument, distribution, activation, degradation and excretion of the insecticides is also being investigated.

Biological control research is being performed to determine the value of natural agents as supplementary control measures. On dairy cattle, materials



and techniques of application are being tested for their effects on weight gain, milk production, milk contamination and animal health as well as pest control. Milk and tissues are being recovered from treated animals and examined for pesticide content. Detection of metabolites as well as the original compounds is being emphasized.

There are 16.1 professional man-years devoted by the States to research on insects affecting dairy cattle.

#### PROGRESS-USDA AND COOPERATIVE PROGRAM

##### A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies were continued on the biology of Anopheles quadrimaculatus by artificially augmenting the population in an isolated breeding area. It was shown that: (1) the density of the population was increased in the study area when a constant breeding area was supplied to egg-laying females and when a blood source was provided, (2) wild females preferred natural ponds as an egg-laying site but used artificial ponds when natural areas were not present, and (3) Anopheles quadrimaculatus adults preferred resting boxes which were painted black and placed on a horizontal plane.

New rearing diets and rearing techniques were evaluated for Anopheles quadrimaculatus. Rye grass infusion or extract in the rearing medium accelerated larval development. There was a positive correlation between the number of larvae per rearing pan and larval mortality. Protozoans were an important source of food for the larvae. A rearing method incorporating all of these factors increased survival and size of the insects and decreased the time required for development and their tolerance to insecticides. Similar results were obtained with four other species of mosquitoes, Aedes aegypti, A. taeniorhynchus, Culex quinquefasciatus, and Aedes triseriatus.

A new method of separating pupae of Anopheles quadrimaculatus from larvae has been adopted. When mixtures of both are placed in ice-water, larvae sink and pupae float allowing rapid separation through the use of a funnel. Time required to separate the stages in colony production was reduced 86%.

At Corvallis, Oreg., studies were continued on the biology of the mosquito, Aedes increpitus, in the Willamette Valley. During the winter months of the last three years, larvae of this species have been collected in numerous habitats of the flood plain of the Willamette River. Following an unusually protracted period of subfreezing temperatures during which a low of 8° F was registered and near-record floods during which all low lying areas in the Willamette Valley were inundated for several days, larvae could be readily collected. The strain of increpitus in the Willamette Valley apparently has become well adapted physiologically to the rigors of the area over a long term period of time. In other studies, a strain of Culex pipiens



quinquefasciatus, which is orange in color as 4th-instar larvae and newly formed pupae, has been isolated. The strain has bred true for 3 generations and appears to be genetically recessive.

At Lake Charles, La., basic studies on the biology of floodwater mosquitoes was conducted. Studies have shown the comparative longevity, blood-feeding, and oviposition patterns of different species of flood-water Aedes species. Aedes taeniorhynchus, A. sollicitans, and A. infirmatus are more important as pest mosquitoes than other species that occur in the area. Studies on the amount of blood ingested by 12 pest mosquito species occurring in Southwestern Louisiana indicated that females of all species ingested sufficient blood at one feeding to at least double their body weight. Psorophora cyanescens, Aedes atlanticus-tormentor, and Anopheles quadrimaculatus, more than tripled their body weight with blood from one feeding.

Research has been conducted under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the production, relative abundance and dispersal of pest mosquitoes in the Gulf Coast area of Louisiana. Data has been maintained on rainfall and temperature in relation to mosquito production and some information has been obtained on the influence of rains versus tidal action in mosquito production. An impoundment is being developed to study the effects of impounding and water management procedures on mosquito production in the area.

2. House Fly. At Gainesville, Fla., research was continued on basic biology of the house fly. Evidence was obtained that an olfactory attractant, or pheromone, specific for the males of Musca domestica L. is not produced only by the females. The attractant was found on contaminated holding cylinders and on dead and non-virgin females. In addition, live males were also somewhat attractive. The degree of attraction was of a low order, resembling other reported sex pheromones of the house fly. This attractant was soluble in methane and slightly soluble in benzene. Data also showed that the time of day at which pupal eclosion occurs is influenced by photoperiod, but photoperiod may not be the only controlling factor. Response to insecticides was also shown to be regulated by photoperiod.

In mating experiments, female house flies mated more readily with males from their own strain than with those of other strains. When normal females from the laboratory or Grand Turk (wild) strains were confined with normal males from one strain and chemosterilized males from the other, they mated more readily with males of their own strain, whether sterilized or not. Chemosterilized males competed more successfully than normal males of the same strain.

At Corvallis, Oreg., research was conducted on the genetics and physiology of house flies and data developed in these studies were used to elucidate mechanisms of insect resistance to insecticides.



Several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. Several of the mutant strains have been defined genetically and are being maintained. Most mutants involved wing form, wing positioning, or pattern of wing venation. Three established mutant strains, classic wing, stubby wing, and dot vein have proved useful in the genetic analysis of insecticide resistance. For example two DDT resistant strains of house flies were found to possess a fifth chromosomal dominant which confers moderate resistance to DDT, but does not confer resistance to o-chloro DDT. In addition, one of the strains possessed a second chromosomal recessive which conferred moderate resistance to DDT and high tolerance to o-chloro DDT. The presence of both factors in a strain confers virtual immunity to DDT. Substrains were isolated, each possessing only one major factor for resistance and the nature of the two independent factors for resistance confirmed through appropriate crosses and bioassays. Resistance associated with the fifth chromosomal dominant is that for DDT dehydrochlorinase. The mechanism of resistance associated with the second chromosomal recessive is unknown, but apparently does not involve dehydrochlorination.

Physiological studies of mechanisms of resistance to organophosphorus insecticides in house flies showed that blocking of Ali-E with a selective inhibitor increased the accumulation of paraoxon and also the toxicity of parathion and paraoxon in both susceptible and parathion-resistant strains. These results indicated that Ali-E is an important detoxifying enzyme. The role of Ali-E in organophosphate poisoning appeared to be related more to detoxication of paraoxon than of parathion in both susceptible and parathion-resistant house flies.

A resistance factor (esterase(s)) for parathion in house flies was shown to be transmitted in a dominant manner and was at least 1000 times less sensitive to inhibition by paraoxon than a corresponding esterase present in a susceptible marker strain.

In other studies successful mating of house flies kept in complete darkness from the time of emergence from pupae to separation of the sexes occurred. Sex ratios were normal among the offspring.

3. Stable Fly. At Gainesville, Fla., studies were continued on the stable or dog fly, a serious pest of animals and humans along the northwest coast of Florida. A general outbreak of stable flies occurred during the last week of August and the first 2 weeks of September in 1964. Population counts around motels and other structures and on beaches ranged from landing rate counts of 2 to 94 flies per man per minute. Bay grass deposits in 1964 were much heavier in 1964 than in 1963. Observations indicated that the insects developed in the bay grass in about the same length of time as was required for development in CSMA medium under laboratory conditions.

Tests indicated that the effectiveness of insecticides against stable flies varied with the time of exposure after blood feeding. Other research in



large outdoor cages indicated that landing rate counts of stable flies were a good index of total populations since the same percentage of flies landed on human observers when the total number of flies was varied.

In Nebraska the exposure of successive generations of stable fly larvae to DDT in the larval medium resulted in a gradual increase in tolerance to the insecticide. By the 39th generation tolerance had increased by 45-fold, but by the 42nd generation tolerance had declined to about 2-fold. The reasons for this rapid decline have not been determined.

In Texas studies were conducted to determine the effects of 4 different conditions of light (continuous light or dark, 12 hours light and 12 dark, and normal daily fluctuation) on the pattern of emergence of adult stable flies from pupae. Emergence in all tests was essentially circadian in pattern, with peaks between 6 and 9 a.m. The bulk of emergence in each group occurred on the 3rd, 4th, and 5th days after first emergence.

In Texas studies were conducted to determine the function of the antennae in feeding and mating behavior of male and female stable flies. When the antennae were completely covered with Plexiglass glue the flies did not feed and mating was greatly reduced. Additional tests showed that blinding the flies by covering the ocelli with black paint also greatly reduced mating. These results indicate that the antennae play an important role in feeding and mating responses of flies and that sight (i.e., light) is necessary for maximum mating response. Further studies showed that when stable fly pupae were held in total darkness, adults emerged and fed but only a small percentage mated and produced viable eggs.

4. Face Fly. In Nebraska, laboratory studies on the reproduction of the face fly showed that as the proportion of males to females was increased, reproduction per female decreased. This was true when only fertile males were mated with the females as well as when various ratios of fertile and sterile males were used. Decreased reproduction apparently was due to harassment by males which reduced female longevity and thereby reduced oviposition.

Studies were conducted under laboratory conditions to determine the egg-laying pattern, number and fertility of eggs and longevity of laboratory reared face flies. Female flies were allowed to mate once then confined individually in small cages for observation. Longevity of the flies ranged from 9 to 60 days, averaging 28.4 days. Batches of eggs per female ranged from 0 to 9 and the number of eggs per female varied from 0 to 181, averaging 56.6. Viability varied from 0 to 89.0%, averaging 60.2%. A few flies oviposited when 4 days old but the average age was 10 days. Results of these tests indicate that multiple matings may be necessary to insure sufficient spermatozoa to insure fertilization of all eggs laid.

Preliminary studies with 3 different strains of face flies showed very little difference in the longevity, fecundity, and rate of development of



the colonies maintained under continuous light and under 16 hours of light and 8 hours of darkness. No circadian rhythms were observed but studies are being continued.

In Nebraska studies were conducted to determine the pattern and distance of dispersal of marked, laboratory reared flies released in the field. Observations indicated that the flies dispersed rapidly in all directions. Maximum distance of recovery from the point of release was 1.4 miles after 24 hours. After 10 days a few marked flies were observed at the release site.

Face flies were first observed in the field in May but populations remained low until late July, whereas in 1963 high populations developed by mid-June. Populations on cattle remained fairly constant in August but fluctuated with changes in temperature in September. When temperatures were below 70° F very few flies were active. Maximum populations of 13 per cow were noted on September 29, a warm day, but on this date flies began to hibernate in one location (grain storage building) although the inside temperature was 90° F. However, the flies moved in and out of the building and small populations persisted on cattle until the advent of cool weather in late October.

Studies were continued in 1964 on the insect fauna in cattle droppings on 3 of 8 farms studied in 1963. Over half of the insects collected in droppings were Diptera, 42% of which were face flies. The total insect population in 1964 was substantially higher than in 1963 but the number of face flies in droppings was almost identical. Parasitism of face fly pupae was low (0.7%) in 1964 but adult populations on cattle were constantly lower than in 1963. Reasons for this were not determined. In addition to face flies, droppings contained substantial numbers of aphodius beetles and Sarcophaga larvae. About 16% of Sarcophaga pupae were parasitized by Hymenoptera and Staphylinidae. Collections showed 10 species of Diptera, about 30 species of Coleoptera and 3 species of parasitic Hymenoptera.

In Maryland, outdoor behavior of face flies was studied, both on herds in the field and on a single animal confined in a cage with a known fly population. The data indicates the following: Only a small proportion, usually less than 10-15% of the total face fly population actually annoys cattle at any given time. Female flies visit the animals much more frequently than males, but males do cause some annoyance. Three- and five-day-old females visit the cow more frequently than 1-day-old females. The number of flies present on cattle is most closely related to the activity of the animal, with the greatest annoyance when the cattle are resting quietly. Although flies visit the face most frequently, they do rest on other parts of the body in considerable numbers. An evident peak of activity of released colonized flies occurred early in the morning, but that of wild flies appeared more evenly throughout the day. All flies leave the cattle at dusk while natural light levels are still quite high.



Additional studies of the nocturnal habits of face flies confirmed that they rest on the foliage of trees at night. Although the flies are readily attracted (about 80%) to blacklight in confined spaces, those found resting on foliage at night do not respond in this manner. Electrocutor grid traps with black light lamps placed in trees attracted less than 1% of the released population in 48 hours. Investigations of the factors affecting this change in behavior may provide information useful in control.

Laboratory tests of the mating activity of female face flies indicate that sterilized males compete effectively with normal males in mating. Also, females appear to mate only once if they are inseminated during their first mating. Examination of females observed attempting to remate showed that only 5 - 10% of all females had not received any sperm during their first mating. This characteristic indicates that use of sterilized males should be effective in preventing reproduction. A laboratory trial using a ratio of 8 sterilized males to 1 untreated male to 1 untreated female resulted in a 94% reduction in pupae.

When marked face flies were released near cows in Maryland about 1 hour before sunset, a few marked flies and a few wild flies were noted on the cows for several minutes after release. They left the cattle when the level of natural light was relatively high, indicating that artificial light would probably not be an effective attractant. In dispersal studies, 24,000 individually marked face flies were released in 4 different areas. Several marked flies were found 2 miles from their release point after 24 hours. One fly was found 4 miles away after 5 days. Searchers for marked flies during the late fall showed that they spend the night in trees and tall weeds, rather than in or around barns.

5. Horn Fly. In Texas, a number of adult horn fly diets were compared for suitability in maintaining laboratory colonies. Diets composed of bovine blood, a saline extract of ground beef muscle and antibiotics or bovine blood, ACD, and cholesterol proved most satisfactory. Flies consumed more, reproduced better and survived longer on these than any other diets tested, including the standard (citrate blood, tissue fluid and antibiotics). Liquid extracts from manure of cattle fed fresh oat and dry alfalfa, sorghum and prairie hay were poured over cotton gauze pads and compared for suitability as horn fly larval media. The oat and alfalfa extracts produced pupae that averaged 3.16 mg in weight as compared to 2.5 mg and 2.1 mg for those reared on sorghum and prairie hay extracts, respectively. In additional tests in which the pH of the media was adjusted, horn fly larvae survived and developed equally well when pH's ranged from 6.0 to 9.0 but none developed in pH's 5.5 or less or 9.5 and above.

In Texas, studies showed considerable variations in the color of eggs deposited in manure by horn flies. Counts indicated about 84 percent of the eggs were dark brown, 13 percent tan and 3 percent yellow and white. The eggs were equally viable and equally capable of producing progeny.



In Texas, pressuring of successive generations resulted in a gradual increase in adult tolerance to ronnel. By the 20th generation a dosage of 0.1 µg/fly caused only 32-56 percent mortality. By the 28th generation tests showed an LD 50 of 0.3 µg/fly or about 50 times that of a normal susceptible strain.

6. Screw-worm. Research was continued on the screw-worm fly at Mission, Tex., in support of Southwestern screw-worm eradication program. Special studies were continued to develop a strain of flies resistant to starvation. Continuous selections resulted in a gradual increase in resistance to starvation until only negligible mortalities occurred in 72 hours in the 19th generation, in 96 hours in the 36th generation and in 144 hours in the 40th generation. Substrains removed from selection in the 18th and 30th generation retained their ability to withstand starvation. The sexual vigor of starvation resistant flies decreased gradually as resistance increased but the substrain removed from selection showed almost normal vigor 7 and 9 generations later. When females of the 27th generation of the resistant strain were held with males of the same strain under 96 hours of starvation no viable eggs were produced. When females were fed the hatch of eggs was 38% as compared to only 14% when only males were fed. When both sexes were fed hatch was 57% or about the same as that for fed unselected females and males.

Studies were continued on the mating behavior. It has been generally believed that female screw-worm flies mate only once, however, close observations showed that a high percentage of the female flies that mated with 1-day old males (immature) mated a second time with mature males. On the other hand only 2 of 110 females first mated to mature males remated, 1 willingly and the other under duress. Egg viability was only 24% when females were mated with 1-day old males but increased to 65% when females were remated with mature males. When 1-day old males were exposed for 8 hours with 3-day old (mature) females and then replaced with mature males egg hatch was only 52%, compared to 89% for matings of mature males and females. These results indicate that females seldom remate if their first mating is satisfactory, i.e. with a mature male.

Competitiveness of irradiated (R) and non-irradiated (N) males was compared in multiple mating of females. Frequency of remating was increased by subjecting females to starvation periods of 20 to 24 hours and all matings were confirmed by observation. Females mated first with R males and then with N males averaged 33% to 48% fertility in 2 tests, compared with 75% to 85% for the controls. However, in the reciprocal matings fertility was 80% in both tests, indicating that R males were not competitive with N males. Mortality and fecundity of starved controls subjected to single matings were normal; however, 33% to 71% of the remated females failed to survive. Actual cause of death following forced second matings has not been determined but these observations help to clarify SAG test results in which aggressive males greatly accelerate female mortality.



Occasionally mating and fecundity studies have been conducted in which oviposition was induced immediately after copulation. Since duration of sperm storage in some mammalian females greatly affects fertility, this possibility was investigated in screw-worms. In 2 out of 3 tests fertility ranged from 47% to 55% when oviposition occurred within 3 hours of copulation, compared with 78% to 86% at 24 hours. In a third test fertility was 59% within 4 minutes but ranged from 80% to 97% from 3 hours to 4 days. Although the influence of sperm storage on fertility has not been clearly defined, a minimum 24-hour period between copulation and oviposition has been adopted in mating and fecundity studies.

Three screw-worm males selected at random from the Florida colony mated a total of 72 females each. All matings were confirmed by observation during daily 4-hour sessions. Two males ceased mating at 7 days when excessive wing damage appeared to interfere with proper positioning, and the other was dead on the 7th day. Peak mating activity occurred during the 3rd to 5th days. Total matings ranged from 1 on the 1st day to a maximum of 20 on the 3rd day; however, from the 3rd to 6th days fertility primarily occurred only among the first 7 matings. Total females fertilized per male (hatch 1% to 98%) ranged from 18 to 24. These results are in agreement with previous studies in which mating activity was evaluated only on the basis of hatching records. Although female remating seldom occurs following copulation with a mature, spermatous male (unless the females are too weak to elude the male), almost 70% of the females in the above test remated.

Studies were conducted to compare the ovarian growth of normal and starvation-resistant female flies under starvation, feeding after starvation and continuous feeding. Under starvation very little growth occurred but when food was provided the starvation-resistant females showed faster development than those of the normal strain. Ovarian growth was comparable when the two strains were fed continuously. Ovarian growth was more rapid in females fed meat than in those on a meatless diet.

Bioclimatic studies showed that both normal and selected strains of newly emerged screw-worm flies survived well when held 30 to 45 hours at 32° F, mortalities being 25 and 10 percent, respectively. Oviposition and egg viability of surviving females was not affected by the exposure. Exposures of 48 to 72 hours at 105° F caused 46 and 38% mortalities of the two strains and oviposition by the survivors and viability of eggs was greatly reduced.

Laboratory studies showed that male screw-worm flies reared on horse meat were about 25 percent larger (based on weight) than flies reared on the liquid medium now used for mass production of screw-worms for use in South-western control program. Also the meat-reared males were able to mate with 40% more females than the liquid-medium reared flies, although no difference was observed in the number of mating attempts by the two strains.

A preliminary investigation made in January in Mexico indicated that in average winters there is very little screw-worm overwintering in the north-



western corner of Sonora or in the northern part of Lower California. However, in the coastal regions of Sonora between parallels  $28^{\circ}$  and  $30^{\circ}30'$ , it is seldom cold enough to completely eliminate screw-worms. Instead they are confined to certain bowl-shaped terrain features known locally as bahias. These bahias are warm and moist and furnish preferred pasture for cattle both winter and summer. It appears that the bahias have somewhat the same relation to screw-worm survival as the river valleys in southwest Texas, but it is not known to what extent screw-worms move from one bahia to another at different times of the year.

In Texas field tests were conducted to study the relative dispersal abilities of irradiated normal and selected (starvation-resistant) strains of screw-worm flies. A total of 330,000 flies of each strain were distributed by airplane in two releases along a 6-mile swath on a large improved range area. Totals of 1659 selected strain flies and 2252 normal flies were recovered from traps, indicating that the normal flies were more vigorous than those of the selected strain.

In August 1964, a test was initiated in a 2000-square mile area in Veracruz, Mexico, to determine the efficiency of dispersal of flies dropped from aircraft at 8-mile swath intervals as compared to the standard intervals of 2-4 miles. Eleven releases of 400 males per square mile were made between August 29 and November 6. Since the area was naturally infested with screw-worms, efficiency was based on the percentage of sterile egg masses collected on wounded animals in pens located 0.1, 2 and 4 miles from release lines as compared to that on animals in a control area. Fly traps were operated at each pen from October 19 until December 1 to obtain data on the relative abundance of wild and released flies. The number of egg masses was fairly high early in August but declined rapidly with the onset of hot-dry weather and remained fairly low until late September. The numbers of egg masses began increasing with favorable weather early in October and remained fairly high until termination of the test. A few sterile egg masses were collected after the first male release. The percent sterility increased steadily thereafter to a peak of 68 percent by October 2, and declined gradually to about 20 percent at time of the last fly drop on October 27. There were no significant differences in egg mass sterility at different distances from lines of dispersal, indicating that the flies dispersed uniformly and apparently rapidly. All trap catches showed larger numbers of sterile flies than wild flies from October 26 through November 6 but native flies outnumbered sterile flies in all traps by November 14. Only 2 sterile flies were present in collections on November 19 indicating a maximum longevity of about 3 weeks.

7. Cattle Grubs. In Texas studies were continued to develop laboratory techniques for the rearing of cattle grub larvae. Several media consisting of agamma calf serum combined with various chlorides, glucose and other materials proved highly satisfactory for in vitro rearing of cattle grub larvae. Some first instar larvae survived as long as 120 days in these



media and many doubled or tripled their weight during this period. Small numbers molted to the second instar and survived another 60 days.

A major problem in in vitro rearing of cattle grub larvae is the development of bacterial infections which kill the larvae and considerable effort was devoted to the development of ways and means to solve this problem. Ultra-violet light effectively controlled bacteria but caused high mortalities of grub larvae. Various antibiotics and merthiolate were partially effective but reduced longevity of larvae. Studies are continuing.

Studies were undertaken to determine the absorption and ingestion of food by 1st instar cattle grub larvae confined in artificial media containing  $P_{32}$ -labeled phosphate. Examinations showed that the larvae contained much higher concentrations of radioactivity in the blood and integument than in the gut at any given time interval, indicating that most if not all intake was by absorption. Similar results were obtained with media containing dyes or carbon black. Dissections of larvae and examinations of the foreguts and hindguts failed to reveal any evidence of a lumen.

In Texas all grubs from the backs of a number of cattle imported from Wyoming were extracted and indentified. The population consisted of 83% H. lineatum and 17% H. bovis.

8. Horse Flies and Deer Flies. In Mississippi, studies were conducted to develop a suitable medium for the rearing of tabanid larvae in the laboratory. Sand and soil was unsatisfactory because larvae did not survive and develop well and they could not be observed without removing them. A semi-solid agar medium proved fairly satisfactory but it tended to harden with age. Of other media tried the most satisfactory consisted of small glass beads covered with water. The larvae survived well, were able to move freely, the container and larvae were easily cleaned, and the larvae were easily observed while moving and feeding on fly larvae or worms. From 60 to 85 percent of larvae of T. americanus, T. abdominalis, T. proximus, and C. crepuscularis survived by this rearing technique. Survival of 4 other species was much lower. Larvae of T. vittiger schwardti showed the most rapid development from egg to mature larvae. Length of larval instars ranged from 5-6 days for the 1st, 2nd, and 3rd to 52 days for the 7th. The average time from egg to mature larvae was approximately 120 days. Attempts to mate adults were unsuccessful.

In additional tests larvae of T. vittiger schwardti developed from the 1st through the 4th stage fairly rapidly in from 5.0 to 11.3 days per stage but development of succeeding stages required successively longer times, ranging from 18.3 days for the 5th to 60.5 days for the 8th stage. Length of the pupal stage ranged from 5 to 10 days, averaging 7 days. Total days from egg to adult ranged from 143 to 209 days, averaging 189 days.

In Mississippi, after many unsuccessful attempts a method was developed for obtaining eggs from engorged adult tabanids. Engorged adults are placed in



a screen cylinder which is then inserted into a wide-mouth gallon jar containing 2-3 inches of water-saturated sand covered with a screen shield. The upper end of the screen cylinder projected 4-5 inches above the mouth of the jar and is covered with cheesecloth. This arrangement provides the range in humidity necessary to satisfy the different water requirements of various species. With this arrangement eggs were obtained from 8 species and records made of the numbers of egg masses laid and days to hatch. The number of egg masses ranged from 1 for T. atratus and 4 other species of 10 for T. abdominalis. Eggs of T. lineola and T. vittiger schwardti hatched in 2-3 days but those of other species required from 5 to 7 days. Newly hatched larvae of most species survived and developed well in thin layers of agar in plastic dishes.

In Mississippi, adult horse fly populations increased gradually during the spring, reached a peak during late May and early June, and declined rapidly during late June and early July. Seven species were present during this period but the predominant species was T. vittiger schwardti followed by T. lineola, T. fuscicostatus, T. equalis, T. abdominalis, T. americanus and C. crepuscularis.

## B. Insecticidal and Sanitation Control

1. Mosquitoes. In Oregon 56 compounds were screened on cattle by the "spot test" method for effectiveness as toxicants and repellents against adult mosquitoes. None of the materials were outstanding toxicants. Four materials--ENT 27194, ENT 27195, ENT 27196 and ENT 28086--showed fair to good repellency at dosages of 500-1000 mg/ft<sup>2</sup>.

At Gainesville, Fla., the developmental program on insecticidal compounds for mosquitoes was continued. A large number of new candidate materials were tested in the laboratory for their potential as larvicides and adulticides. Many proved promising for further development.

Tests were conducted to evaluate fogs of naled, fenthion, Bayer 39007, and malathion against caged salt-marsh mosquito adults. Bayer 39007 was the most effective in these tests followed by fenthion, naled, and malathion. A field test in which different formulations of malathion were applied by airplane at a ratio of 0.05 lb/acre showed a reduction of 81% in population levels with the fog oil formulation, 76% with fuel-oil formulation, and 44% with water emulsions. Airplane sprays tests with four organophosphorus insecticides on adult salt-marsh mosquitoes indicated all were highly effective at low dosage rates. Comparison of the effectiveness of aerial sprays of malathion applied as a thermal fog and fuel oil spray showed the latter to be more effective.

Tests were conducted in the rice-growing area near Stuttgart, Ark., to evaluate the residual effectiveness of some new insecticides against natural infestations of Anopheles quadrimaculatus. The insecticides were applied to the walls and ceilings of farm buildings at 200 mg/ft<sup>2</sup> as wettable powders and/or emulsions. Pre- and post-treatment counts were made of the mosquitoes



resting in the treated buildings as well as in six untreated buildings which were utilized as checks. An emulsion of Hercules 9485 was highly effective, causing 99.7%-100% reductions for at least seven weeks. As this compound was not available at the beginning of the series, it was applied from two to three weeks after the other treatments. A wettable powder formulation of Shell SD-8530 caused reductions of 98-100% for 9 to 10 weeks. A malathion wettable powder used as a standard caused 100% reductions for 4 weeks and 96-100% reductions (avg. 99.0%) throughout the 9-week test period. Hercules 9326 emulsion caused 100% reduction of the mosquito infestations for at least 9-10 weeks in two buildings but in a storage shed produced only 91% to 96% control the 6th and 8th weeks. Wettable powder formulations of CELA S-1942 and CELA S-2225 were slightly less effective, with control falling below 70% in some buildings by the sixth week. Shell SD-8211 was highly effective in two buildings but not in a third.

Tests were also conducted to evaluate the residual effectiveness of treated cheese-cloth when applied to the walls and ceiling of buildings in the same area. The cheese-cloth, which was purchased in rolls 3 feet wide, was first flameproofed and then impregnated with Bayer 39007. Buildings in which a complete coverage of treated cheesecloth had been used showed 100% reduction of the mosquito populations for the full 10-week duration of the test. All buildings treated by means of a strip of cloth around the edge of the ceiling and in the corners showed 100% reduction for 5 weeks, and 82 to 99% control for the next 5 weeks. Buildings treated by means of cloth around the edge of the ceiling only, or in the corners only, showed 98 to 100% reduction of mosquitoes for 5 weeks, and 84 to 100% control for the next 5 weeks.

At Corvallis, Oreg., tests were continued on the development of more effective insecticides for mosquito control. In field tests against snow-water Aedes mosquito larvae, excellent results were obtained with lindane, BHC, and fenthion at 0.05-0.1 lbs/acre. Abate and Dursban were generally less effective. Against mosquito breeding in log ponds, granular formulations of fenthion and abate gave excellent control. Both were also effective when applied with a pump oil can. In cooperative tests in California low volume airplane sprays of malathion and fenthion showed considerable promise as mosquito larvicides.

Infusions and hot water extracts made from several tree species were tested for toxicity against Culex tarsalis larvae. Toxic elements were found in Western red cedar, ponderosa pine, and to a less extent in lodgepole pine and redwood. Similar hot water extracts made from Douglas fir, Sitka spruce, Western hemlock, big leaf maple, red alder, and white fir were nontoxic to larvae. Studies are in progress to characterize the toxic principles through fractionation of extracts.

None of 7 analogs of DDT showed promise against resistant Culex tarsalis larvae.



At Corvallis, Oreg., experiments with  $C^{14}$ -TDE indicated that both susceptible and DDT-resistant Culex tarsalis larvae detoxified TDE by dehydrochlorinative and oxidative routes. The results suggested that resistance to DDT and related compounds in tarsalis involves a mechanism other than dehydrochlorination.

Studies were continued in the search for compounds that would act as synergists to overcome insecticide resistance in mosquitoes. Of a number of phosphorus esters, butyl-containing esters were most effective although other types showed activity.

2. House Fly. At Gainesville, Fla., research was conducted on the development of safer, more effective insecticides. Materials were evaluated in the laboratory as contact sprays and residual toxicants as a basis for selecting promising insecticides for field evaluations. Evaluations as residual toxicants included different formulations of the materials. Twenty-one promising compounds were tested as house fly larvicides in manure under caged poultry. Four compounds were highly effective as larvicides.

Residual tests were conducted with emulsions of malathion, diazinon, ronnel, dimethoate, naled, fenthion, and Bayer 41831, and with wettable powders of malathion and Mobil MC-A-600 against house flies in barns. All were applied at 100 mg/ft<sup>2</sup>. Control was considered satisfactory as long as the reduction produced by the chemical was 75% or above. Dimethoate residues gave satisfactory control on most occasions for 14 days, after which they were ineffective. Mobil MC-A-600 wettable powder gave satisfactory control for 14 days in one test, but failed as early as the 1st day in a replication of the test. Other compounds gave satisfactory control for shorter periods.

At Corvallis, Oreg., research was continued to find compounds effective in synergizing organophosphorus insecticides and resistant strains of house flies. A number of different types of phosphorus esters were effective when combined with either malathion or parathion in overcoming resistance in house flies to these two compounds. Materials synergizing malathion against resistant insects differed considerably from those known to potentiate the toxicity of malathion to mice or cause ataxia in poultry.

3. Stable Fly. In Texas 119 new compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Nine of these compounds were Class IV toxicants at concentrations of 0.5 percent or lower. Materials effective at a low concentration of 0.1 percent were Shell SD-8967 and SD-9102, and Cela S-1942. The only effective repellents at a 5% concentration were ENT-nos. 28086, 28087 and 28093. Special tung oil formulations from a commercial source proved both non-repellent and non-toxic to stable flies. Spot tests were conducted to evaluate a number of materials as extenders for pyrethrum and conventional insecticide. One material, Armour ARD-226, increased the repellency of malathion and extended residual effectiveness slightly. Other materials were ineffective.



In Texas, large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low volume sprays for the control of stable flies. Treated cattle were exposed for 24 hours periodically to flies in large cages but otherwise were kept outdoors. Pour-ons (8 oz/animal) of coumaphos were effective against stable flies for 15 days. Pour-ons of fenthion and ronnel were only slightly less effective, but carbaryl was effective for only 3 days. Conventional 2-quart spray applications of 0.1 percent Stauffer R-5723 and 1 percent Mobil MC-A-600 and Cela S-1942 were effective 3 to 10 days against stable flies. In similar cage tests with low volume sprays (23-69 ml/cow) 1 percent applications of 0.5 percent methoxychlor, malathion and DDT controlled stable flies for 3-6 days as compared to 1-3 days for 7 other materials.

Comparative tests were run with the WHO test kit to compare the susceptibility of stable flies to 12 insecticides. Five of the materials were equal in toxicity to ronnel (standard) and 3 were more toxic, namely, Shell SD-8436, SD-8447 and SD-8448.

At Gainesville, Fla., chemicals were evaluated in the laboratory as potential larvicides for the control of stable flies. Approximately 150 compounds were tested by exposing larvae to these compounds when they were incorporated into the larval rearing medium. Approximately 20 of the compounds were highly effective, approximately equal in activity to a standard, Bayer 39007. Tests with calcium arsenate as a larvicide indicated that it did not compare favorably with other compounds that were evaluated. In addition approximately 120 compounds were evaluated in laboratory tests as adulticides and some 20 were highly effective. Several of the more promising adulticides were tested as fogs against caged adults under field conditions, indicating the potential of these materials for controlling natural populations. Comparative tests of adulticides against caged insects indicated no differences in effectiveness of thermal vs. non-thermal fog applicators or between fuel-oil and water-based formulations. A contract was negotiated with the Florida State Board of Health to conduct research on insecticides for controlling natural populations of stable flies under conditions found in the Gulf area of Northwestern Florida. The research contract will take advantage of research conducted at the Gainesville Laboratory and evaluate insecticides under practical field conditions.

4. Face Fly. In Nebraska bioassay tests showed that the addition of 1.0 ppm of Thiabendazole, a new parasiticide for livestock, to manure prevented the development of face fly larvae to the adult stage. Concentrations of 0.1 ppm and lower were ineffective. The addition of 0.5% of Bacillus popilliae to manure had no effect on the development of face fly larvae but the addition of 1.0% reduced fly emergence by 24%.

5. Horn Fly. In Texas large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low volume sprays for the control of horn flies. Treated cattle were exposed periodically for 24 hours to flies in large cages but otherwise were kept outdoors.



Pour-ons (8 oz/animal) of coumaphos were effective against horn flies for 20 days. Pour-ons of fenthion and ronnel were slightly less effective and carbaryl was effective for only 3 days. Conventional 2-quart spray applications of 0.1 percent Stauffer R-5723 and 1 percent Mobil MC-A-600 and Cela S-1942 were effective for 7-10 days. In comparative tests low volume sprays of 5 and 10 percent ronnel gave 100 percent kill of horn flies in 24 hours whereas 1 percent sprays gave only 86 percent kill.

In Oregon large cage tests were conducted to determine the effectiveness of certain tacky polybutanes against horn flies. All of the materials were repellent the first day after application but only one--Amoco H-120--showed repellency for 2-3 days. Additional cage tests were run to evaluate the effectiveness of 1/2 inch, 1 inch and 1 1/2 inch plastic collars containing 20 percent dichlorvos for the control of horn flies. The collars reduced horn fly populations by 86 to 100% in 3 hours and 100% in 24 hours and were still completely effective after 2 weeks of wear and exposure.

In the mid coastal areas of Texas pour-on applications of 2 ounces per cow of 8% Ruelene and 5% ronnel gave excellent control of horn flies for 6 days, while applications of 4 ounces were effective for 11 days. In central Texas conventional sprays of 0.3 percent Ciodrin, 1 percent trichlorfon, 1 percent Cela S-1942, 0.5 percent carbaryl and a pour-on of 1.0 percent coumaphos provided effective control for 2 weeks. Several other materials were effective for about 1 week. Similar treatments gave slightly shorter periods of control in humid coastal areas.

Low volume sprays of 5 and 10 percent ronnel applied to 1 square foot areas (withers and brisket) gave good control of horn flies but lower concentrations were unsatisfactory. In other tests excellent control of horn flies was obtained by treating only part of the animals in herds with 8% Ruelene at 1 oz/100 lbs body weight as a pour-on treatment. In one test the treatment of only 2 cattle in a herd of 50 reduced the overall horn fly population by 75 percent in 10 days.

In Texas bioassays were run to determine the toxicity to horn fly larvae of the manure from cattle that had been fed seven insecticides at varying rates for 10 days. All of the materials reduced larval survival but only Bayer 37341 and Stauffer R-3828 at 5 mg/kg daily gave 100 percent mortality.

In Mississippi, conventional spray applications of 2 quarts per cow of 0.375 percent coumaphos, 0.2 percent Bayer 9017, 0.5 percent Hooker 1422 and 0.5 percent methoxychlor provided effective control of horn flies for 10 days. The lowest test concentrations of 0.06 percent coumaphos, 0.06 percent trichlorfon, 0.05 percent Bayer 9017 and 0.1 percent fenthion were effective for 6 to 8 days or about as long as 2- to 4-times higher concentrations. In comparative tests back rubbers treated with 0.25, 0.5 and 1.0 percent Bayer 9017, 0.5 and 1.0 percent coumaphos and 0.5 percent ronnel maintained effective control of horn flies for 7 to 8 weeks, whereas those treated with 0.5 percent dimethoate and 0.5 percent Famophos were effective only 4



weeks. Observations suggested that loss of effectiveness was due to loss of the oil solvent since the odor of insecticide was still detectible. Retreatment of each backrubber with 1 gallon of oil resulted in 4 weeks additional control, thus confirming the above observation.

In Mississippi, a series of tests were run to compare the effectiveness of 0.5 percent oil solutions of 10 insecticides applied in low volumes by automatic sprayers. Single applications of ronnel, Ciodrin, Dioxathion, Bayer 9017, Shell compound 4072 and Dowco 175 provided excellent control of horn flies for 6.5 to 8.5 days. Other materials were equal or slightly less effective than toxaphene (standard) which gave satisfactory control for 5 days.

6. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Of twenty new compounds screened for larvicidal effectiveness at 10, 1.0 and 0.1 ppm in screw-worm larval medium, four were highly effective, killing all the larvae at 1.0 ppm, namely, Shell SD-8964, Shell SD-8988, Shell SD-8967, and Geigy GS-12968. None of the compounds screened were effective at 0.1 ppm.

In field tests in Mexico, cattle infested with 1- and 2-day-old screw-worm larvae were sprayed or dipped in promising insecticides. Shell Compound 4072 in a dip or spray at 0.1% killed all the larvae, as did Cela S-1942 in a 1.0% spray. Hooker HRS-1422 as a 0.25% spray and Shell Compound 4072 as a 0.08% in a dip were fairly effective but permitted a few larvae to survive. Telodrin as a 0.05% spray killed both 1- and 2-day-old screw-worm larvae but it also killed 3 of the 4 cattle treated. Bayer 37289 (0.25% spray) and Bayer 38333 (0.1% spray) killed all the larvae in one test, but not in another. Sprays containing 0.1% of ethion, 0.1% of Dowco 175, 0.05% of dimetilan, 0.01% of Bayer 29952, 0.01% of Stauffer N-2790, or 0.01% of Bayer 38156 were ineffective.

Previous research has shown that sprays containing 0.1% or higher concentrations and a dip containing 0.1% of Shell Compound 4072 are effective screw-worm larvicides. In new tests in Mexico, cattle with wounds containing 1- and 2-day-old screw-worm larvae were dipped in vats containing either 0.05% or 0.1% Shell Compound 4072. At examination 24 hours after treatment, no live larvae were found in wounds on cattle dipped in 0.1%. All 1-day-old larvae were killed by 0.05%, and live 2-day-old larvae were found in only 1 of 16 wounds.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas 113 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Ten materials showed systemic activity in one or both types of administration. The most effective materials, dosages and routes of administration were as follows: Shell SD-9129, 5 mg/kg,



O and SC; Spencer S-6900, 25 mg/kg, O and SC; and Cela S-2225, 25 mg/kg, O. Seven other materials were effective at dosages of 50 to 200 mg/kg.

In Texas field tests were conducted on small numbers of Government-owned cattle (2 to 4) to evaluate the effectiveness of a number of materials that had shown promise in screening tests and of several older effective materials administered in different ways at several dosages. Materials giving 91-100% control of grubs when administered in the feed for 10 days were as follows: Bayer 37341 and Bayer 37342, 1.0 mg/kg; and Famophos, menazon, and Vamindoate, 5.0 mg/kg. As drenches, Cela S-1942 at 100 mg/kg and Shell SD-8949 at 50 mg/kg gave 91 and 100% control, respectively. Menazon as a 1.0% spray gave 100 percent control. Other materials were ineffective.

In Texas field tests were conducted on cooperative cattle on 9 ranches with several experimental materials and with a number of older systemics administered at different dosages in several types of formulations. In pour-on tests materials, concentrations, formulations, and the lowest rates of application that gave 95-100% control were as follows: 4.0% oil suspension of coumaphos, 10 mg/kg; 15.5% water emulsion of Bayer 37342, 100 mg/kg; 4.0% water emulsion and oil suspension of Ruelene, 25 mg/kg; 7.75% water solution of trichlorfon, 50 mg/kg; and 10.2% oil suspension of ronnel, 150 mg/kg. Conventional spray treatments giving 92-100% control of grubs were 0.25% fenthion emulsion, 0.5% Rulene water suspension, 70 mg/kg, 0.25% Shell Compound 4072 water suspension, and 1.5% trichlorfon water solution, 250 mg/kg.

In Texas field tests were conducted to evaluate the effectiveness of 6 materials on Wyoming cattle infested with the northern cattle grub, Hypoderma bovis, as well as the common cattle grub, H. lineatum. Oral administration of Stauffer R-3828 at 25 mg/kg gave 90% control of grubs. The other materials were partially or completely ineffective.

In Oregon extensive field tests were conducted to evaluate the effectiveness of 7 insecticides as pour-ons and 2 as sprays for the control of cattle grubs. In these tests, pour-ons of 8% Ruelene in water emulsions at 52 mg/kg and in oil solutions at 25, 34, and 46 mg/kg per animal gave 99% control of grubs. Similar results were obtained with pour-ons of 8 and 12% trichlorfon at 30 and 45 mg/kg, and 2% fenthion at 7.5 mg/kg. Pour-ons of ENT 25482 at 91 mg/kg and Shell SD-8436 at 12 mg/kg gave 97% control of grubs and Shell SD-8447 at 121 mg/kg was only slightly less effective. Sprays of 0.1 and 0.25% Imidan® showed 88-89% control but lower concentrations were ineffective.

8. Horse Flies and Deer Flies. In Mississippi, sprays of 2 percent Ciodrin gave excellent immediate protection of cattle from horse flies but no effect was apparent after 3 hours. Applications of 1 percent Ciodrin plus 0.25 percent dichlorvos provided excellent protection for 7 hours and up to 24 hours in some tests.



9. Ticks. Studies were continued in Texas to develop effective systemics and conventional insecticides for use in the control of several species of ticks on cattle and other animals. A total of 114 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larval lone star ticks. Only 6 of the materials showed systemic effectiveness. The outstanding materials, dosage and method of administration were as follows: Velsicol FCS-13, 25 mg/kg, O and 10 mg/kg, SC; and Spencer S-6900, 25 mg/kg, O and SC. The other 4 materials were effective at dosages of 50-100 mg/kg by one or both methods of administration.

In Texas 160 insecticides screened in dipping tests against engorged Boophilus females to determine their effectiveness in preventing oviposition and/or hatch of eggs. A total of 44 of the insecticides were ineffective at the highest test concentration of 1.0%. The remainder were effective at 1.0% or lower concentrations. Materials that were effective at the lowest test concentration of 0.01% in preventing oviposition were as follows: Shell SD-8448 and SD-9102, and Niagara NIA-9227. Materials which permitted light oviposition but prevented hatching of eggs were as follows: carbophenothion, Stauffer R-2964, N-3727 and N-3794, Monsanto CP-40272 and Wm. Cooper 57-H-62.

Extensive field tests were conducted in Mexico to evaluate the effectiveness of 12 promising insecticides as sprays and/or dips for the control of Boophilus ticks on cattle. In dipping vat tests, concentrations of 0.05-0.1% of Shell Compound 4072 gave 100% mortality of flat and engorging stages and no live ticks were noted after 1 week, indicating that residual material killed all molting stages. Similar results were obtained with sprays of 0.1% Shell Compound 4072 and Dowco 175 and 0.01% Bayer 38333. Sprays of 0.25% Hooker HRS-1422, 0.1% ethion, 1.0% Cela S-1942, 0.25% Bayer 37289, and 0.01% Bayer 38156 were highly effective but a few ticks were still alive on treated animals after 1 week. Sprays of two materials--0.05% Teleodrin and 0.01% Bayer 29952--killed or severely poisoned cattle. Dimetilan and Stauffer N-2790 were not highly effective.

In field tests in Texas, sprays of 0.1% Shell Compound 4072 and fenthion, 0.25% Imidan and 0.5% Cela S-1942, malathion and toxaphene gave highly effective control of the winter tick, Dermacentor albipictus, on cattle. Little or no reinfestations developed on treated animals within 1 month after spraying.

In field tests in Texas, sprays of 0.25% coumaphos and Imidan, 0.03% diazinon, 0.1% Shell Compound 4072, 0.3% Ciodrin, 1.0% trichlorfon, and 0.5% toxaphene gave excellent immediate control of lone star ticks on cattle. Four other materials failed to give satisfactory control. Pour-on applications of 8.0% trichlorfon and 2.0% Hercules 7522, which are excellent systemic treatments against cattle grubs, were relatively ineffective systemically against ticks.

Extensive field tests were conducted in Texas to evaluate the effectiveness of 32 insecticides as sprays and/or dusts for the control of spinose ear tick, Otobius megnini, in the ears of cattle. All of the materials except Dri-Die and Dowco 175 dusts, and menazon sprays gave excellent to complete control of infestations of the spinose ear tick. However, the only treatments still showing effective control after 1 month were 5% dusts of coumaphos and Shell Compound 4072, 1% Hercules 7522 dust and 0.3% Ciodrin spray.

Small-scale field tests were conducted in Florida to evaluate several insecticides for effectiveness against the tropical horse tick (Dermacentor nitens), the vector of equine piroplasmosis. In these tests dermal and ear applications of 1% lindane, 0.3% Ciodrin, 0.25% Imidan and coumaphos, and 0.1% Shell Compound 4072 gave 100% immediate control of ticks but light reinfestations developed in all instances in 2 weeks. Dri-Die dust was ineffective. In systemic tests trichlorfon at 10 mg/kg a day for 10 days was completely effective in clearing ticks from the ears of horses. Fenthion at 5 mg/kg for 5 days, Famophos at 10 mg/kg for 4 days and Hercules 7522 at 5 mg/kg for 4 days reduced but did not completely eliminate tick infestations.

Surveys in southern Florida showed D. Nitens to be present at 4 of 15 locations examined but populations were high in only one. Insecticidal treatment of pastures and horses apparently have eradicated the tick in the other 11 locations.

10. Lice. In Mississippi 14 promising insecticides were evaluated by the spot test method against cattle lice. Three materials, Stauffer B-10046, R-5724, and R-5725 gave 100% immediate kills of motile lice but all permitted reinfestations to develop in 14 days. In field tests, two applications of 5% dusts of coumaphos, carbaryl and dioxathion 2 weeks apart eliminated lice on cattle. Similar applications of 5% methoxychlor gave excellent control but did not completely eliminate the lice.

In Nebraska, treatments of groups of cattle with 0.5% ronnel applied with a Bean Rotomist sprayer eliminated all motile stages of cattle lice. However, after 1 month light infestations were again present on some animals.

### C. Insecticide Residue Determinations

1. Residue studies. In Texas tests were conducted to determine the levels of residues in tissues of cattle forced to use back rubbers treated with 1 and 2 percent ronnel-oil solutions four times daily for 28 days. Small average residues of 0.005 to 0.05 ppm were found in the fat after 2 weeks but only negligible amounts were present after 4 weeks and none whatever could be detected 2 weeks after treatments were discontinued. Residues in muscle, liver, kidney, heart, brain, and spleen were barely detectable after 2 weeks treatment and none whatever could be demonstrated thereafter.



Additional studies were conducted to determine the distribution of  $p^{32}$ -ronnel dermally on cattle forced to use backrubbers treated with this material. Comparisons were made between animals receiving 2 and 4 exposures per day for 4 weeks. Distribution as indicated by analyses of hair samples was very irregular but most of the insecticide was concentrated along and adjacent to the central back line and tip of the head. Cattle treated 4 times a day received about twice as much insecticide as those treated 2 times daily. No ronnel was present on the hair 2 weeks after the last treatments.

In Texas two tests were conducted to determine the sites of accumulation and amounts of residues in various tissues of cattle resulting from dermal sprays of 0.1 percent Shell Compound 4072 emulsion. In one test, the cattle were sprayed weekly for 12 weeks; in the other test they were sprayed 6 times at 2-week intervals. In the 12-weekly spray test, analyses 1 week after the first spray showed residues in the fat ranging from 0.007 to 0.045 ppm (average 0.02 ppm). Residues increased slightly with successive weekly sprayings to a peak average of 0.14 ppm (range of 0.097 to 0.196 ppm) after the eighth spraying. One week after the twentieth and last spraying residues in the fat averaged only 0.01 ppm (range 0.008 to 0.016 ppm). No residues were detectable 2 weeks after the last spraying. In the biweekly spray test analyses 2 weeks after the first and third sprays showed only 0.005 ppm in the fat but residues increased somewhat after the fifth and sixth sprays, averaging 0.117 and 0.133 ppm, respectively. Additional analyses of fat from animals slaughtered 2 weeks after the sixth and last spraying showed average residues of 0.065 and 0.112 ppm in renal and omental fat, respectively. No residues were detectable 4 weeks after the last spraying.

Analyses of tissues from a calf slaughtered 7 weeks after being sprayed with 0.25 percent Imidan showed no residues in samples of fat, muscle, heart, liver or spleen.

In Maryland, as a result of the detection of residues of heptachlor epoxide in the fat of cattle slaughtered at the Agricultural Research Center at Beltsville, analyses were made to determine if residues were present in the milk of three experimental dairy herds. The over-all average residue of heptachlor epoxide in the milk was below 0.01 ppm. Analyses of eight lots of hay and one lot of alfalfa pellets which were being fed to the cattle showed residues ranging from 0.00 to 0.048 ppm of heptachlor epoxide. Subsequent tests did not show detectable residues in the feed but low levels of heptachlor epoxide persisted in the milk of selected animals from experimental herds.

2. Toxicity Studies. Research was continued in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals to livestock.

Studies were conducted to determine the normal patterns of certain enzymes in average cattle as a prerequisite for studying the effects of chemical



poisoning on enzyme patterns and the effects of oxime-type cholinesterase reactivators (2-PAM, DAM and TMB-4). Cattle poisoned by Dioxathion caused elevations in activity of serum glutamic oxalate, pyruvate transminases, alkaline phosphatase, and blood beta lipoprotein but these increases were minimized by the administration of 2-PAM and TMB-4. All three test oximes prevented decreases in gamma globulin. These results indicated that TMB-4 was slightly more beneficial than 2-PAM. DAM did not appear beneficial at the levels (10-20 mg/kg) tested.

Cattle were poisoned with an oral dosage of dichlorvos to determine the effects on serum glutamic oxalacetic and pyruvic transaminase, aldolase and alkaline phosphatase. Oximes were given some of the cattle to determine their protection of those enzyme systemics. DAM and 2-PAM kept the enzyme activities of the mildly poisoned animals near normal, whereas TMB-4 appeared to cause an increase in activity above normal during the test. From the biochemical standpoint, it appeared that 2-PAM and DAM offered more protection to the enzyme activities than does TMB-4.

Cattle were poisoned with coumaphos and enzyme systems studied in serum. Some of the cattle received antidotal therapy with 2-PAM. Glutamic dehydrogenase, sorbital dehydrogenase, phosphohexose isomerase and serum arginase were studied in an effort to find significant enzyme activity alterations indicative of possible tissue change. No significant differences were noticed in the enzyme activities regardless of treatment. Mortality was reduced by 2-PAM in coumaphos poisoned animals, but this benefit could not be detected in the enzyme studies.

Additional studies were conducted to determine the effectiveness of these three oximes in reversing cholinesterase inhibition induced by organic phosphorus compounds. Each of the compounds was useful, but 2-PAM and TMB-4 appeared to be superior to DAM. Particularly encouraging was the beneficial effect of these compounds in cattle poisoned by coumaphos; usually such animals do not readily respond to atropine, the pharmacologic antidote. Oximes combined with atropine markedly increased the number and speed of recoveries.

Studies were conducted to determine the effects of the chemosterilant, apholate, when fed to Jersey cattle at 1 mg/kg daily throughout one gestation period. A deficiency of white blood cells appeared in one heifer after 80 daily doses and in the others after 110. One heifer died after 335 doses, one delivered a calf and died after 531 doses, one delivered prematurely and survived 581 doses, and the fourth delivered at term and survived 629 doses. In additional studies the new chemosterilant, hempa, appeared to be considerably less toxic than apholate, tepa and metepa, but it produced the same deficiency in white blood cells.

Studies were conducted to determine the toxicity of 72 insecticides to cattle, sheep and goats. Toxicities ranged from impossibly dangerous to



reasonably safe. Promising new insecticides showing low toxicities were Shell SD-8447 and Cela S-1942.

#### D. Biological Control

1. Mosquitoes. At Lake Charles, La., research on pathogens of mosquitoes has been conducted. Field collections throughout the area have shown infections of microsporidia in thirteen species including the genera, Culex, Aedes, Culiseta, Anopheles, and Orthopodomyia. Aedes grossbechi and Orthopodomyia signifera were new host records for a microsporidian. Spore sizes were determined and classification of the microsporidia studied. Transovarial transmission of microsporidian infections was studied in 12 species of mosquitoes and demonstrated in seven of the species.

The fungus Coelomomyces was found in field collections infecting larvae of Culex restuans, C. salinarius, Aedes vexans, A. sollicitans and Culiseta inornata. Culex alinarius, C. restuans and Aedes sollicitans represent new host records for Coelomomyces. Field infection levels varied from very low to over 50 percent.

A polyhedral virus was reported from larvae of Aedes vexans and Psorophora ferox. Both species were previously unreported as hosts of viruses. Infection levels in the field were very low. A very lethal bacteria was collected from larvae of six mosquito species.

At Gainesville, Fla., two species of Thelohania were found in A. quadrimaculatus, one infecting the adipose tissue and the other the oenocytes. One species was found infecting the oenocytes of A. crucians larvae.

2. Face Fly. Studies in 1963-64 by personnel of the European Parasite Laboratory, Insect Identification and Parasite Introduction Research Branch, in France showed that the adults of Aleochara tristis, a staphylinid beetle, were predaceous on larvae of the face fly and other Diptera breeding in cattle droppings and that newly hatched larvae parasitize face fly pupae. Shipments of this species were received at Lincoln, Nebr., in January and February 1965 and immediate steps were taken to establish colonies. The adult beetles were caged under room conditions (80-85° F; 50-60% RH) and provided fresh cattle manure containing face fly eggs and small larvae. The beetles survived well, oviposited and newly hatched larvae were noted in 12 days. Face fly pupae were made available to the small larvae. Observations showed that the larvae quickly penetrated the face fly puparia and closed the entrance hole. The larvae consumed the face fly pupae and pupated within the puparia. Adult beetles emerged from the puparia through the area normally used by emerging flies. The combined larvae and pupal period was about 17 days and the adult preoviposition period of about 12 days. Large numbers of adults will be produced and released at selected field sites near Lincoln and studies made to determine the effectiveness of this parasite in reducing face fly populations.

3. Horse Flies and Deer Flies. In Mississippi, large numbers of tabanid larvae were collected periodically and transported to the Kerrville, Texas, laboratory to determine the number and species parasitized and the identity of the parasites. A total of 206 larvae, representing 4 species, were collected in October 1964. Only 3 larvae of T. atratus showed microsporidian infections. In subsequent collections in November 1964 and January 1965, microsporidian infections were found in some of 3 species of tabanid larvae, namely, T. sulcifrans, T. vittiger schwardti and T. atratus. The microsporidia in sulfifrans could not be identified but those infecting vittiger schwardti were of the genus Plistophora. Those infecting atratus were Thelohania sp. Healthy atratus larvae readily became infected when fed spores of Thelohania but not when fed those of Plistophora. Microsporidian infections could not be induced in fed or starved healthy larvae held at 10° C. At 25° C fed larvae readily developed infections but not starved larvae.

In Texas, approximately 300 tabanid larvae were collected and examined during the year and all were free of microsporidia and other parasitic infections.

Studies were conducted to determine the host range of microsporidia (Thelohania sp.) by feeding artificially infected C. macellaria larvae to different species of tabanid larvae. The normal host of this parasite, Tabanus atratus, readily became infected. Of 5 other species used, only one, T. americanus, developed typical spore infections. Similar studies were made with another microsporidian (Plistophora) but results were negative.

In Texas efforts to develop a reliable spore agglutination test based on sedimentation patterns formed by spores mixed and allowed to settle with rabbit antisera were unsuccessful. In other tests antimicrosporidian spore-rabbit serum reacted positively by agglutination against homologous antigen but further tests must be conducted to ascertain the specificity of the reaction.

4. Ticks. In Texas laboratory tests were conducted to determine the effectiveness of a dust preparation of the fungus Beauveria bassiana against 4 species of ticks. Adults and nymphs of Amblyomma americanum, adults of A. maculatum and adults of Dermacentor variabilis were highly susceptible to the fungus with 90 to 100% kills occurring in 7 to 10 days. Nymphs of the spinose ear tick Otobius megnini were not affected.

#### E. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., studies were continued on factors affecting the attraction of mosquitoes to their hosts and factors affecting the protection time from mosquito bites afforded by repellents. A large olfactometer was developed to study these factors as well as evaluate the efficacy of various attractant materials or factors.



The effort to develop effective space and systemic repellents was continued and slightly expanded. To date several materials show some space repellency to mosquitoes in that they prevent mosquitoes from penetrating 4-mesh screening. Materials exhibiting some systemic repellency were found and further tests will be made on these materials.

Studies were continued at Gainesville to evaluate materials as chemosterilants for mosquitoes and to evaluate the sterility principle of mosquito control. Tests with hempa indicated it would have little value as a residual sterilant for mosquitoes. Further selection and studies were conducted with the apholate-resistant colony of Aedes aegypti to clearly define the degree of resistance. This colony is at least 10 times as resistant to the sterilizing effects of apholate as the unselected, parent colony. Selections to increase resistance will be continued.

A sterile male release study of Anopheles quadrimaculatus was made in a semi-isolated area. This site was made more favorable by increasing the number of breeding sites and introducing additional wild stock of this mosquito to populate the area. When sterile males of wild stock were released in this area sterility of the natural population increased from a very low degree up to 42%. When the releases were changed to sterile colony males the sterility in the natural population decreased confirming earlier work showing behavior differences between colony and wild strains in seeking out wild females. Apparently a sufficient number of males was not released to reduce the population levels of A. quadrimaculatus.

Studies were continued on the evaluation of chemosterilants for mosquitoes at Corvallis, Oreg. The chemosterilant, hempa, was not highly effective in sterilizing Culex tarsalis larvae in that rates as high as 200 ppm were required. As a residual treatment in glass jars, 10 mg of hempa per square foot sterilized adult males completely, but adult females only partially. In wind tunnel tests against adults, a concentration of 10% caused high sterility, whereas a 5% spray caused only partial sterility and none was caused at 1% or lower. Males were generally more susceptible than the females.

At Corvallis studies were continued on sex and ovipositional attractants for mosquitoes. Preliminary tests indicated the presence of sex attractant in Culex quinquefasciatus, but further tests did not confirm its presence, nor the presence of a sex attractant in Culex tarsalis. Many mosquitoes are known to choose specific types of water for oviposition. Studies have shown that odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of Culex quinquefasciatus. These odors were not attractive to females of C. tarsalis. However, log pond water itself was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of C. quinquefasciatus but

not those of C. tarsalis. Gravid females of both species were more attracted to distilled water treated with 25 ppm of furfural than to water treated with 5 or 50 ppm.

2. House Flies. At Gainesville, Fla., research was continued on the development of chemosterilants and the sterility principle of control for house flies. Several hundred new candidate compounds were evaluated in primary screening and secondary development tests and many were found to exhibit sterilizing efficacy against both males and females. Particular attention was paid to evaluating two compounds--hempa and hemel--as sterilants by several routes of administration. These two materials will sterilize both sexes of the house fly.

Two series of field tests were conducted at farms in Florida to evaluate the effectiveness of two chemosterilants, hempa and apholate, for the control of house flies. At the farm treated with hempa, house flies were reduced in abundance from 71 per grid to 0 within eight weeks. Grid counts remained at zero for the remainder of the test period. At the farm treated with apholate, population levels decreased from 200 per grid to less than 10 within 6 weeks and remained constant at a low level throughout the remainder of the test.

Ninety chemicals were screened as chemosterilants against adult house flies. Six compounds were toxic and 10 reduced the fertility to some extent in fly food or sugar. Thirty-two compounds, previously shown to sterilize house flies, were also tested again at higher or lower concentrations.

Tests were conducted with 21 compounds to determine their effectiveness as male house fly sterilants. Of the 14 chemicals Olin 53330, Squibb Olin 53331, Squibb Olin 53356, and Squibb Olin 53263 sterilized at a concentration of 0.05% in the sugar diet. Sankyo Co. RES-101 induced sterility at this dosage in sugar in the first two eggings.

Basic studies were continued on the cytological effects of chemosterilants on house fly reproductive systems and previous sectioning, fixing, and staining techniques have been used to study several new chemosterilants.

Olfactometers designed by Gouck and Schreck were used to initiate a search for more effective house fly attractants. Nineteen compounds were tested as house fly attractants, using Edamin as a standard. Beef protein concentrate was attractive to females but not to males. The other materials were not as effective as Edamin.

At Corvallis, Oreg., research was conducted on chemosterilants and attractants for the house fly and the little house fly. With the little house fly, hempa caused sterility as a residual deposit on glass at 50 mg/ft<sup>2</sup>. However, the sterilizing dose caused some fly mortality. Higher doses were highly toxic to the adult flies; lower doses did not sterilize. When fed orally in the adult food to the little house fly, hempa was toxic at 0.25%



and lethal at 1.0%. High, but incomplete sterility was caused at concentrations as low as 0.01%. Topical treatments of hempa and hemel sterilized males of the little house fly without causing mortality, but not the females. In general treatments causing a high degree of sterility did not affect the mating competitiveness of females. Four known antioxidants exhibited little effect on egg production or egg hatch with the little house fly. Dosages of gamma radiation greater than 1000 r given to pupae of the little house fly prevented oviposition by emerging adults.

At Corvallis research was continued on the sex pheromone in house flies. The presence of a low titre of pheromone in extracts of pupae and young females was shown and confirmed. Higher activity of the pheromone was demonstrated in 3-day-old flies. The presence of the pheromone was shown in female flies from strains of different origin. Males of different strains reacted to extracts from females of different strains, though differences in behavior were apparent.

At Corvallis, Oreg., further studies showed that the sex pheromone in female house flies increased with the age of the flies, with the greatest increase occurring on about the third day after emergence. Comparative tests with benzene extracts of female house flies showed that high concentrations applied to pseudo flies inhibited male response. Similar inhibition occurred when normal extracts were applied to large surfaces within test chambers. Comparative tests with extracts of females with different solvents showed a much greater amount of the pheromone in hexane than in other solvent extracts. Efforts are being made to determine the chemical nature of the pheromone.

At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of house flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

3. Stable Fly. At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of stable flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

4. Face Fly. At Beltsville, Md., additional tests of the responses of 3-day-old female face flies to monochromatic light confirmed that blacklight



is highly attractive under conditions of confinement and that wavelengths in the red and yellow spectral regions are unattractive. Refinements of the testing procedure and methods of data analysis are being made in an attempt to more clearly establish differences in attractiveness.

5. Horn Fly. In Texas, topical applications of 1 µg/fly of apholate sterilized adult horn flies. Lower dosages of 0.5, 0.2 and 0.1 µg/fly reduced the hatch of eggs but did not confer completely sterility. Males were more easily sterilized than females. In feeding tests, flies feeding over-night on a diet containing as little as 0.01 percent apholate were completely sterilized.

6. Screw-worm. In Texas 20 of 255 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worms when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration; some sterilized only one sex, and some sterilized both male and female flies. A review of past chemosterilant screening revealed that of the aziridine compounds screened, 92 were effective either by multiple-oral administration or topical application, or both. Only 8 were less than 100% effective when administered orally. In the future, routine use of topical applications in chemosterilant screening will be dropped in favor of multiple-oral administration.

It has been shown that there is a differential susceptibility between males and females sterilized with metepa. Tests with uredepa (ENT-50450) showed similar results, with the males about 9 times more susceptible than females on the basis of dosage/unit of body weight. There was also a greater variation in results obtained with females than with males. Starvation also increased the effectiveness of uredepa. The antifertility effects of another chemosterilant, ENT-25296, were enhanced by subjecting treated flies to periods of temperature stress (98° or 14° F), after administration of sterilizing or highly effective substerilizing dosages.

Male and female screw-worm flies can be sterilized by exposure to certain chemosterilants, but most of these chemicals adversely affect mating activity, longevity, or vigor. Three new chemosterilants were found that equal or surpass radiation in their effectiveness in achieving sterility of screw-worms. ENT-50838 applied topically provided a wide margin of safety between the minimum toxic quantity and the sterilizing dosage. Males sterilized with this material were hypercompetitive; they were sexually more aggressive than irradiated flies. The other two compounds, ENT-50716 and ENT-50781, were more toxic to the flies, but they were approximately equal to radiation as sterilizing agents.

In Texas 154 chemicals and other materials were screened as screw-worm attractants. Of these, 22 were equal to or better than the standard liver bait and require further evaluation. Some have been tested in the field in limited tests. The most outstanding were isovaleraldehyde, ethyl



isovalerate, and an ethanol extract of the flowers of Yucca treculeana. Liver-baited traps were seldom as effective as traps containing these materials. The presence of blooming wild flowers interfered with these tests; tests made during peak blooming seasons usually gave negative results.

The presence of a pheromone produced by males that is attractive to virgin female screw-worm flies was confirmed. In Texas, by means of the cold-trap method, 3500 ml of condensate were collected over a 5 1/2-month period from a cage containing virgin male screw-worm flies. Benzene and chloroform extracts of the condensate were capable of changing the behavioral pattern of virgin female flies. Sexually mature females in the presence of the odor go through "searching" motions and finally behave as in an aggressive mating "strike". Young (1-2 days old) females gave little or no response to the male odor, but 3-day-old females exhibited a definite activity, including the imitation of "male-type strike". Four-day-old females reverted to the response of 1-day-old females, but activity increased again in 5-day-old females. The greatest response was observed in 6-day-old females, with 7- and 8-day-old females showing a decrease in total number of "strikes". This decrease may have been due to wing damage, normal at this age. Other studies are in progress, including fractionation of the extracts to find the effective material. Another extract, made by filtering the air in the eradication colony room, brought a response on the part of both males and females.

7. Ticks. In Texas studies on the effects of radiation on lone star ticks showed that dosages of 250 and 500 r did not affect fertility of adults treated 1 week after molting from nymphs. However, a dose of 500 r sterilized females 1 day after engorging. Doses of 250 and 500 r had no effect on engorging and molting of nymphs or on fertility of resulting adults. However, at 1000 r the percentage molting of nymphs was reduced and resulting adults did not engorge or lay viable eggs.

#### F. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas and Mississippi in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the State Experiment Stations to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. In Texas, monthly surveys were made to determine the identity and abundance of external parasites on infected and clean (segregated) herds of cattle. Lone star tick populations were light on cattle in January, increased gradually during February, March and April, were heavy from May through July and then declined rapidly to insignificant numbers by September. Winter ticks first appeared late in October, increased gradually to peak abundance in late December, then declined to insignificance by March. The spinose ear tick was present throughout the year, with populations being moderate to high at practically every examination. Cattle lice populations were extremely low throughout the year.



Horn flies appeared in April, increased steadily to moderate numbers by June, remained at this level until late September, and disappeared with cool weather during late October and early November. Light to moderate grub infestations were present in the back of cattle from early January until mid-March. Some transmission continued to occur in the infected herd but the segregated herd remained clean. It was thus demonstrated that young calves removed from the presence of carrier cows could be maintained anaplasmosis free. The test was discontinued this year.

In Mississippi studies on the relative importance of day-and night-feeding insects in the transmission of anaplasmosis, which were initiated in 1963, were continued in 1964. In the main test, three groups containing negative, splenectomized steers were used, one group being exposed continuously, one during the day only, and one at night only. The test period of 6 weeks (June 1 - July 13) was divided into three 2-week intervals and during each interval one other group of cattle of the same composition was exposed continuously in hopes of pinpointing the 2-week period in which most transmission occurred. Each group was exposed daily with animals infested with anaplasmosis.

During the first two weeks (June 1-13) horse flies were moderately abundant, but declined in numbers abruptly and only small numbers were present the last 3 weeks of the test. Populations of horn flies were relatively low and stable flies relatively high throughout the test period. Mosquitoes were fairly numerous the first week of the test but populations declined thereafter and remained low throughout June. Populations rose rapidly early in July and were high during the last two weeks of the test. The mosquito population was largely Psorophora confinnis until the last week of the test, when Anopheles quadrimaculatus increased abruptly to make up about 40 percent of the population.

In the main 6-weeks test, two cases of anaplasmosis developed in each of the continuous- and day-exposure groups, but none occurred in the night-exposure group. In the other groups exposed continuously for 2 weeks, one case each occurred in the first (June 1-16) and second (June 16-29) exposure periods but none developed in the third (June 29-July 13). These results indicate that anaplasmosis transmission most likely occurred during June when horse flies were most abundant. This indication is supported by the fact that no transmission occurred in the cattle exposed at night when only mosquitoes attacked them. However, additional studies are needed to clarify the role of mosquitoes as possible vectors and to determine whether one or more species of horse flies is capable of transmitting the disease.

At Beltsville, Md., studies on the transmission of bovine anaplasmosis and the development of the disease organism in experimental vectors were continued in cooperation with personnel of the Animal Disease and Parasite Research Division.



Efforts to colonize a "Nevada line" of Dermacentor andersoni were unsuccessful. The larval progeny from a single engorged female received from Nevada did not attach and feed on the test calf, and all died.

An adult-to-larva hereditary transmission trial with Dermacentor occidentalis was negative. Several thousand larvae attached and fed on the test calf, but anaplasmosis was not transmitted. The calf was proved susceptible by inoculation with Anaplasma infected blood.

Studies were conducted with Dermacentor occidentalis to determine whether or not the ticks can become adapted to infection with Anaplasma marginale by feeding one or two of the developmental stages in each generation on calves acutely infected with A. marginale. The plan of study was to feed adults on infected calves, feed the resulting larval progeny on guinea pigs and then test part of the ensuing nymphs on a susceptible calf. If transmission did not occur, the remaining nymphs and succeeding adults were to be fed on acutely infected calves and tested again in the following generation. In the first generation studies the adult-to-nymph trial was negative and the test calf proved susceptible by challenge. Approximately 1,000 nymphs were allowed to feed on an infected calf and 50 of the resulting adults were tested on a susceptible calf for a trans-stadial transmission. This experiment has not yet been concluded. The remainder of the D. occidentalis adults, infected as nymphs will be fed again on an infected calf and tested again as 2nd generation nymphs.

Systematic studies on Anaplasma-infected and non-infected D. occidentalis salivary glands are being conducted concurrently with each transmission experiment.

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## II. UTILIZATION RESEARCH

### DAIRY PRODUCTS - CHEMICAL, PHYSICAL AND BACTERIOLOGICAL CHARACTERISTICS: DEVELOPMENT OF NEW AND IMPROVED PRODUCTS AND PROCESSING METHODS Eastern Utilization Research and Development Division, ARS

Problem. Dairying is one of the largest segments of American agriculture: dairy products represent more than 13 percent of all farm cash receipts; milk production requires about 140 billion feed units annually (1 unit is equivalent in feed value to 1 pound of shelled corn); milk is a highly nutritious food. It is clear from these facts that research which succeeds in increasing the consumption of milk will have far-ranging effects in raising nutritional levels, in increasing farmers' income, and in increasing consumption of feeds. There is opportunity to increase milk consumption, for per capita consumption is currently at its lowest point in over 40 years at 585 pounds, whole milk equivalent. Current consumption in the U. S. is well below that of several foreign nations, including New Zealand, Canada, Australia, Sweden, Norway and the United Kingdom, all using more than 800 pounds per capita.

Increased consumption can result from improved quality of manufactured dairy products, from cost reductions based on improved processing technology, from the development of new products, or from any combination of these. The development of new and improved processes and products is the objective of utilization research on dairy products.

Both basic and applied research in this field are needed; applied research is the direct antecedent to the development of new products and processes and basic research provides the information which permits applied research to proceed most effectively.

Increased emphasis on basic research has been advocated by the Utilization Research and Development Advisory Committee and the Animal and Animal Products Research Advisory Committee. Basic research is considered primarily the responsibility of public agencies which disseminate their findings for use by all.

One aspect of the problem posed by dairy products is the great need for fundamental information on the complex biophysical-chemical system which each dairy product is. The development of new products and new processing technology through applied research represents the exploitation of fundamental information. Such exploitation and development cannot continue indefinitely; the supply of fundamental information must be maintained and enlarged, and this is the purpose of basic research. The complexity of milk makes necessary the employment of several scientific disciplines in basic research on this commodity. These disciplines undertake investigations needed to identify and measure the amounts of individual chemical components present. They also seek to define the molecular structure of these components; how the molecules react; and the forces determining the course of the reactions. These studies should be intensified. Other needed investigations

include: study of the synthesis of milk; the properties of milk fat and of the milk proteins; and the factors responsible for the flavor of dairy products and the changes in flavor which occur during processing and storage.

There is also need for a vigorous and sustained program of applied research which is aimed to increase consumption of dairy products. Such a research program could stimulate consumption by development of products with increased palatability, convenience, or extended shelf life. Another opportunity is the possibility of developing new and improved processing technology which will reduce costs. Applied research is most effectively done by technologists closely associated with basic scientists where cross fertilization of ideas can stimulate both groups.

Increased milk consumption, however achieved, should have a powerful upward effect on feed consumption. Since milk production requires about 140 billion feed units annually, a 1 percent increase in milk production would require feed equivalent to 25 million bushels of corn--the production of some 400,000 acres. If the feed were supplied by cropland pasture, almost a million acres would be needed.

It is thus manifest that utilization research on dairy products can provide a powerful stimulus to American agriculture.

#### USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, microbiologists, food technologists, and engineers, engaged in basic research on the composition and properties of milk, and in applied research directed to the development of new and improved dairy products and processing technology.

The Department's research facilities are located in Wyndmoor, Pennsylvania, Washington, D. C., and Beltsville, Maryland. The Federal (USDA) scientific effort devoted to research in this area totals 91.5 professional man-years (p.m.-y.), which includes 10.7 p.m.-y. in the domestic contract and grant research program. The effort is distributed as follows:

(a) Work on flavor aspects of dairy products involves 6.0 p.m.-y. at Washington. One category of the flavor studies is identification of desirable flavors and their precursors. Research under a grant (0.8 p.m.-y.) to the University of Maryland, College Park, deals with flavors and flavor precursors in milk derived from pasture or dry feed. Under a grant (1.4 p.m.-y.) to Oregon State University, Corvallis, research is proceeding on the desirable flavor components of butter. A second category of flavor studies is identification and analysis of components responsible for stale flavor. Research under a grant (1.3 p.m.-y.) to Pennsylvania State University, University Park deals with lactones, methyl ketones, and their precursors. In addition, research sponsored by the Department under P.L.-480 grants is in progress at: (1) National Dairy Research Institute, Karnal, Punjab, India, on sulfur compounds in relation to flavor and stability of milk; (2) Biochemical



Institute, Helsinki, Finland, on dietary factors controlling flavor in milk. Research at the Institute of Biochemistry, University of Turku, Finland, on growth-promoting factors for lactic acid bacteria was completed during the year.

(b) Research on whole milk products involves 22.0 p.m.-y. at Washington and Wyndmoor. The program includes fundamental and applied research on development of liquid milk concentrates (6.0 p.m.-y.) and dry whole milk (16.0 p.m.-y.) that will be acceptable to the consumer market in quality and storage stability. In the category of liquid concentrates, a grant to the Ohio State University Research Foundation, Columbus (0.8 p.m.-y.) supports research on the physical state of calcium phosphate-containing casein micelles in the concentrates. A research grant at North Carolina State University, Raleigh, (1.0 p.m.-y.) is concerned with the physical changes associated with steam injection and bubble collapse during milk concentration. Some small-scale consumer testing of dry whole milk is done through the cooperation of the Statistical Reporting Service. In addition to the domestic research on whole milk, the Department sponsors research under P.L.-480 grants at: (1) Technical University Berlin, Berlin-West Germany, on surface-changes in fat globules of dried whole milk; (2) Centro Experimental del Frio, Madrid, Spain, on protein destabilization in frozen concentrated milk; (3) "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, on thermal and related physical properties of milk and milk products.

(c) Basic research on milk involves 34.6 p.m.-y. at Washington and Wyndmoor. These long-range fundamental studies include the following subjects: structures and interactions of casein and other milk proteins; bacterial spores; structure and properties of nucleic acids; influence of genetics on structure of milk proteins (cooperative with the Animal Husbandry Research Division, ARS); heat stability of milks; milk enzymes; relation of diet of the cow to milk composition. Contract research at the University of Minnesota, St. Paul, (0.7 p.m.-y.) deals with the heat stability problem, and contract research at the University of Maryland, College Park, (0.7 p.m.-y.) deals with the relation of milk fat composition to the diet of the cow. In addition, research sponsored by the Department under P. L.-480 grants is in progress at (1) Indian Institute of Science, Bangalore, India, on phosphoproteins of milk; (2) National Dairy Research Institute, Karnal, Punjab, India, on the proteose-peptone fraction of milk; (3) Institut National de la Recherche Agronomique, Paris, France, on nonprotein nitrogenous constituents of milk; (4) Institut National de la Recherche Agronomique, Paris, France, on proteolytic activity of rennin on casein; (5) Centre de Recherches sur les Macromolécules, Strasbourg, France, on structure of nucleic acids in connection with the synthesis of milk proteins; (6) University of Uppsala, Sweden, on methods for purification of protein complexes applicable to milk; (7) National Institute for Research in Dairying, University of Reading, England, on studies on selected enzymes of milk; (8) Instituto Nacional de Tecnologia, Rio de Janeiro, Brazil, on a study of the relation of biological activity of proteins to their structure, as determined by investigations of proteolytic enzymes; and (10) U. P. Agricultural University, Pantnagar, India on dipicolinic acid

synthesis in bacterial spores.

(d) Research on milk fat, cheese and nonfat milk involves a total of 12.0 p.m.-y. in Washington and Beltsville. Study of the production and properties of butteroil involves 4.0 p.m.-y.

Research on improved ripened cheese involves 6.0 p.m.-y.; contract research on the source of Cheddar cheese flavor was completed during the year. Additional research on cheese, sponsored by the Department under P.L.-480 grants is in progress at (1) Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, on potential use of American export nonfat dry milk in manufacture of hard cheese; (2) Institute of Dairy Industry, Warsaw, Poland, on increasing vitamin B content of cheese; (3) College of Agriculture in Olsztyn, Poland, on mechanisms of the cheese-ripening process, and (4) National Dairy Research Institute, Karnal, India, on milk coagulating enzymes.

Research on nonfat dry milk involves 2.0 p.m.-y. at Washington. Contract research at the University of Wisconsin, Madison, (0.3 p.m.-y.) is concerned with the effects of nonfat dry milk on bread yeast.

(e) Research at Beltsville on the identification and removal of radio-nuclides from milk has been discontinued. A research contract with the Producers Creamery Company, Springfield, Missouri, is concerned with development of a commercial scale process for removing radioactive strontium from fluid milk. This contract, supported equally by the Eastern Division and the U. S. Public Health Service, involves a total of 7.3 p.m.-y.

Also, the Eastern Division is supporting jointly with the U. S. Public Health Service contracts with (1) Chemical Separations Corp. on removal of radioactive contamination by use of a moving resin bed; the level of USDA support is 1.9 p.m.-y. and (2) Producers Creamery Company, Springfield, Mo., on removal of radioactive contamination from fluid whole milk by use of a fixed anion-cation resin bed; the level of USDA support is 2.0 p.m.-y.

(f) Pioneering research on the allergens of agricultural products involves 6.2 p.m.-y. at Washington.

#### PROGRAM OF THE STATE EXPERIMENT STATIONS

The research program on dairy products at the State stations includes studies on basic composition of milk and factors responsible for changes in composition. Some primary concerns are isolation and identification of phospholipids and nonglyceride components of milk fat, influence of feed on fatty acid composition, glyceride structure and physical properties of fat, influence of metal-protein complexes on oxidation of fat, synthesis and structure of fat, formation of solid solutions in mixtures of milk fat, and methods for extracting milk complex lipids.

Another phase of the program deals with the physical and chemical characteristics of fat globule membrane proteins; the physical-chemical properties of kappa-casein and its subfractions; the effects of heat treatment on the



structure, properties and interactions of protein components; the change in shape, size and structure due to enzyme action; the role of casein fractions in curd formation; and the influence of protein fractions on the stability of certain dairy products.

Considerable emphasis is placed on enzyme activity in dairy products. Enzyme studies include the susceptibility of milk to lipolysis; the inhibition of lipases by antibiotics; the number and mode of action of lipases in milk; the influence of lipolytic activity on Cheddar cheese; the chemistry of prorennin activation; the microbial enzymes associated with cheese-ripening; and the characterization of lipases from pseudomonas.

Considerable interest has developed recently regarding residues in dairy products. Research is underway to investigate the effects of pesticides on microorganisms used in cultures; lipolysis of milk fat; activity of purified colostrum pseudocholinesterase; the effects of residues on quality characteristics; and the development of techniques for the detection, removal or neutralization of residue effects.

Research on flavor of dairy products includes identification of compounds in cheese responsible for desirable flavor; chemical nature of off-flavors in milk and dairy products; components responsible for natural flavor of milk; effects of rations on oxidized flavor in milk; off-flavors caused by microorganisms; effect of minor organic constituents on flavor; and effects of processing treatments on flavor.

The microbiological phases of dairy products research deal with the physiology of mutants of P. roqueforti, with metabolic activity of heat-resistant bacteria, and with factors affecting the growth of bacteria at refrigerator temperatures. Other phases include flavor production of lactic cultures, and growth stimulants and growth retardants of lactic cultures. The incidence of pathogenic organisms in milk and dairy products and the control of these organisms is under investigation. Other studies include factors affecting rate of acid production, bacteriophages active against lactic streptococci, and the isolation of rennet from microorganisms for cheese-making.

Research is underway on developing improved and new processing technology, and improved product quality for dried, concentrated, and frozen products and for butter. Included in this research are the engineering aspects of automation, physical forces involved in circulation cleaning and packaging, including aseptic packaging.

The total number of man-years devoted to this program is 86.5.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Flavor Aspects of Dairy Products.

In the past year attention has been focused on the identification of

desirable flavor components in milk and dairy products. Contract research at Oregon State University, Corvallis, has demonstrated that a trained panel reliably indicates the acceptability of a beverage milk product by the public. To be acceptable the beverage milk should receive a hedonic score of "6.0, like slightly" or higher. This work demonstrates that a trained panel can be a reliable guide to overall consumer acceptability of the products.

Research under a grant to Oregon State University has provided results indicating preference for samples of butter subject to certain manufacturing variables: from sweet cream, cultured cream, cream heated at 160°F. for one hour, and lipase-induced rancid samples. Progress was made in developing techniques for removing the flavor compounds from butter and identifying them.

Good progress was made in studies of the stale flavor of milk products. It is believed that stale flavor is due in major part to the presence of lactones and methyl ketones, and possibly o-aminoacetophenone, in the product. The lactone precursors are a group of hydroxy fatty acid glycerides and a color reagent, the 2,6-dinitrophenylhydrazone of pyruvyl chloride (DNPHPC) was found to react at room temperature quantitatively and quickly with all types of hydroxyl compounds. Methods have been worked out for separating the DNPHPC derivatives of the various classes of hydroxyl compounds; fractionation of the derivatives from milk fat indicates thus far the presence of at least six types of components. Four of these have been tentatively identified as cholesterol, monoglycerides, 1,2-diglycerides and 1,3-diglycerides. Two components not yet identified may be the glycerides containing hydroxylated fatty acids. The o-aminoacetophenone produced from the nonprotein nitrogen under alkaline conditions appears to be influenced by the initial heat treatment of the milk.

In research conducted under a P.L.-480 grant at the National Dairy Research Institute, Karnal, India, a number of alternative and simple methods have been devised for the quantitative estimation of sulfhydryl groups in milk and its products. These methods are proving helpful in identifying the origin and nature of cooked flavors in heat-processed milk.

Research under a P.L.-480 grant at the University of Turku, Finland has yielded much valuable information on the chemical nature of the factors promoting and regulating the growth of lactic acid bacteria isolated from milk products. Some antagonistic relationships between various amino acids were observed in connection with the nutritional requirements of these bacteria. The maximal enzyme activity level was shown to occur in the exponential phase of growth. This information is applicable for commercial enzyme production and provides a guide for obtaining enzymes of optimal activity.

P.L.-480-supported research at the Biochemical Institute, Helsinki, Finland, is providing new information on the effect of diet on the flavor and quality of cow's milk. Cows maintained on a purified protein-free diet are producing a so-called "zero" milk which serves as a basis for studying the origin of milk flavors. To date, experiments have been concerned mostly with a



comparison of milks resulting from protein-free and protein-rich diets. These milks are strikingly similar in their "normal" taste and smell and in their composition. Analysis indicates that even the protein fractions are similar, although slight differences in fat contents and volatile substances do exist and will be investigated further. Extensive studies on the effect of flavor components in certain fodders, in the rumen contents, and in the cow's blood are now underway. Future findings from this research will be of special value to milk processors, who are extremely interested in the origin and control of flavor in concentrated and dried milks.

## B. Whole Milk.

1. Vacuum foam-dried whole milk. Good progress has been made on the computer analysis of the main effects of the 13 recognized controllable process variables, that is necessary to determine the optimum operating conditions and to achieve year-round control of foam behavior during drying. The mathematical method being used to interpret and extrapolate the experimental data is based on original work published by the DuPont Company, and a DuPont-developed computer program which precisely fills Division needs was made available to the Division without charge. From the analysis, new process conditions are being obtained from prediction equations derived from experimental data. These conditions will deal with overcoming the seasonal foam variation problem at a moderate though economical dryer product rate. If it is found that year-round process control can be achieved, then data analysis and attendant pilot plant studies will deal with maximizing the product rate while maintaining seasonal control.

2. Foam-sprayed dried whole milk. Concentrated milk foamed by injection of air, nitrogen, or CO<sub>2</sub> before spray-drying yields a product of excellent initial flavor during the fall, winter and spring. However, in summer the initial flavor of the foam-sprayed dried whole milk powder is not of uniform high quality. One possible cause of this difficulty with initial flavor may be the high and variable oxidative capacity of atmospheric air during the warm months.

A spectrophotometric procedure has been devised to measure the oxidative change that takes place in the foam-spray drying operation and to trace the origin of the initial off-flavor that appears in the powder sporadically during the summer months. This method is capable of accurately determining peroxide numbers as low as 0.001 and can easily determine the amount of oxidation that occurs in one day's exposure. The cellular structure of foam-spray dried whole milk results in a persistent fine-grained foam on reconstitution, especially when the water is cold. An approach to solving this problem has been to meter liquid carbon dioxide into the high pressure milk concentrate feed. This improved the characteristics of the nonfat dry milk but with whole milk concentrates no improvement was noted.

A small-scale consumer-type test of the flavor acceptability was conducted by the Statistical Reporting Service and showed that the average preference for the reconstituted milk was not significantly different from that for fresh whole milk. A companion test revealed a decided preference for fresh nonfat milk over a reconstituted commercial nonfat milk powder. This suggests that the foam-spray dried whole milk powder approximates its fresh milk equivalent more closely than nonfat milk powders approximate the fresh fluid material.

Research on chemical changes occurring at the surface of the fat globules in stored foam-spray dried whole milk at the Technical University Berlin, Berlin, West Germany, under a P.L.-480 grant, has resulted in the development of reliable methodology for investigating the interactions of the carbonyls present in fat with components in the nonfat portion of the powder particle.

3. Liquid milk concentrates. The establishment by Eastern Division research that polyphosphates are the most effective consistency stabilizers known for HTST sterilized concentrated milk represents a major forward step, yet the mode of action of the polyphosphates is unknown. Evidence was found that polyphosphates in amounts up to 0.1% by weight of the finished product are effective antigelation agents in sterile concentrates because of their action in inhibiting secondary changes that occur during sterilization and storage of the concentrates. It is believed that much of the added polyphosphate is converted to pyrophosphate during sterilization, yet addition of the corresponding amount of pyrophosphate to milk either before or after sterilization does not prevent gelation; in fact, it promotes gelation. Still more surprising is the discovery that 0.2% added polyphosphate promotes gelation. A number of sterile milk concentrates containing added polyphosphates are now either being test-marketed or produced commercially in the United States and the evaporated milk industry, in general, is showing great interest in this new and important discovery. Polyphosphates also have a stabilizing effect in frozen milk concentrates and in sweetened condensed milk. In continuing research on the mechanism of gelation, samples of sterilized whole milk were processed in the pilot plant and stored to determine the effect of processing sequence, preheat treatment, and use of polyphosphates on the extent and character of gel formation and sedimentation.

Also, a whole milk concentrate with 44% total solids was produced and remained fluid for over 3 months. With the control of gelation achieved, more attention is now focused on the sedimentation which occurs in HTST sterilized concentrate; it was found that severe agitation of milk during processing is accompanied by greater sedimentation during storage.

In grant research at Ohio State University Research Foundation, Columbus, fundamental data have been obtained on the nuclear magnetic resonance spectra of some casein complexes. Near pH 7 interactions between polypeptide chains in kappa-casein micelles are strong. In grant research at North Carolina State University, Raleigh, investigations have begun on the durability and response time of thermal sensor probes within a steam injector. Results from these two grants are expected to elucidate some of the storage changes in the concentrated milks that should lead to improved processing methods.



Work under a P.L.-480 grant to the Milan Experimental Station for Refrigeration, Italy, led to the discovery that apple and quince pulps added to milk stabilized the milk proteins so that added stabilizers were unnecessary. Apple and quince pulps gave complete stabilization in many combinations with many different fruits. For example, a stable product that tasted like grape juice was obtained by mixing 0.62 parts of grape pulp, 0.4 parts of apple pulp and one part of skim milk. Experiments were carried out on whole milk, skim milk, and powdered milk and with fresh pulp, canned pulp, and frozen pulp. In general, skim milk and fresh pulp were most satisfactory.

A P.L.-480-supported grant with the Centro Experimental del Frio, Madrid, Spain was completed. These investigators report that neither lactose crystallization nor pH change is too significant in protein destabilization during storage of frozen concentrated milk. The final report suggests that the more important factors are the presence of fat, buffer substances and alginates.

### C. Basic Research on Milk.

1. Characterization and structure of milk proteins. Solubility studies of two of the genetic variants of  $\beta$ -lactoglobulin, namely A and B, were conducted after the removal of two residues of isoleucine and histidine per molecule from each by means of the enzyme carboxypeptidase A. It was found that the removal of these amino acid residues produced a marked reduction in the solubility of both the A and B forms.

Two treatments with carboxypeptidase were required for the complete removal of two residues of histidine from  $\beta$ -lactoglobulin. After these two treatments, both forms had essentially the same solubility in both water and dilute sodium chloride. Thus, the removal of C-terminal isoleucine and adjacent histidine residues eliminated the differences in solubility of the A and B forms even though the modified A form still contained two more residues of glycine and aspartic acid and two less residues of alanine and valine than did the modified B form, this difference in amino acid composition being of genetic origin. The finding that the large difference in solubility between the A and B forms of  $\beta$ -lactoglobulin is eliminated by the removal of the same four amino acid residues from both forms is unexpected and appears to be inconsistent with the idea that the position or properties of the genetic variant amino acids are responsible for the marked differences in the solubility of genetic variant proteins.

In another investigation the electrophoretic patterns of preparations of the red protein isolated from milk of individual cows suggest the existence of a genetically controlled polymorphism in this protein.

The mechanism of the photooxidation of amino acids in proteins sensitized by methylene blue has been further investigated. The light sensitive amino acids--namely, histidine, methionine, tryptophan and tyrosine--and some of their derivatives, were investigated as a function of pH and temperature. These studies led to the proposal of a cyclic-free radical mechanism for the oxidation of amino acids.

Study of the photooxidation of insulin showed that at 10° only two histidine residues are oxidized and that the rate of oxidation is influenced by the shape of the insulin molecule. A direct correlation was found between the destruction of the two histidine residues in insulin and loss of its biological activity.

Good progress has been made in identifying and characterizing enzymes of milk. A fourth ribonuclease (ribonuclease D) has been found. Milk ribonuclease B is a glycoprotein but no carbohydrate has been detected in ribonuclease A. Milk ribonuclease B differs slightly from pancreatic ribonuclease B.

Good progress has likewise been made in fractionating other milk proteins and characterizing them. The amino acid composition of  $\alpha_{S1}$ -A, -B, and C caseins has been completed. Three types of kappa-casein have been found and there is a possibility of a fourth. Since genetic studies on the distribution of the variants of  $\alpha_{S1}$ - and  $\beta$ -caseins are now going on throughout the world, it is important that various laboratories agree on the types observed and the use of the techniques for demonstrating these types. For this purpose eight unknown and one standard sample were distributed to the various laboratories and only one sample was wrongly identified by one laboratory. These results indicate agreement on the type identified and satisfactory performance of the methods employed. Fundamental studies underway at the Institute Nacional de Tecnologia, Rio de Janeiro, Brazil under a P.L.-480 grant have recently provided data which contributes to a better understanding of enzymes which occur in an inactive form and require activation, usually by a proteolytic enzyme before they are functional.

2. Interactions of milk proteins. Study of the solution conformation of milk and other proteins showed that in their native states the three  $\beta$ -lactoglobulins and  $\alpha_{S1}$ -casein contain little or no helical structure while  $\alpha$ -lactalbumin is nearly 40% helical. Since both  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin have highly folded globular structures, ordered structures other than helical must be predominant in  $\beta$ -lactoglobulin. In nonaqueous solvents major structural changes can take place in the milk proteins, for a high helix content can be induced in the caseins and  $\beta$ -lactoglobulin by dissolving these proteins in organic solvents such as acidic methanol and ethylene glycol. Also, the association properties of  $\alpha_{S1}$ -casein C/C at pH's between 2-11 show that this protein is strongly aggregated in acid.  $\alpha_{S1}$ -casein is dissociated completely by solutions of guanidine hydrochloride into monomeric units of molecular weight  $28,400 \pm 2,700$ . A direct correlation has been found between the proline content and amount of helix formation in a number



of milk and other proteins. This was an expected result because proline is known to interfere with  $\alpha$ -helix formation.

In studies of the casein in whole milk concentrates it was found that the effect of hexametaphosphate (HMP) on the caseinate complexes depends upon the concentration of caseinate present. Below a certain critical concentration of caseinate at a given temperature the caseinate is destabilized by HMP. At higher caseinate concentrations the material remains stable for indefinitely long periods at the given temperature. Contract research at the University of Minnesota on the heat-stability of individual milks shows that milk from a cow containing the rare  $\alpha_{S1}$ -casein type A/A had very low heat stability.

Investigations of the proteose peptone fraction of milk under a P.L.-480 grant at the National Dairy Research Institute, Karnal, Punjab, India, has resulted in a publication which describes a simple, reliable method for determining the nonprotein nitrogen fraction of both cow's milk and buffalo milk. Studies on selected enzymes of milk under a P.L.-480 grant to the National Institute for Research in Dairying (University of Reading), Shinfield, Reading, England, have resulted in reliable methodology for fractionating lipases present in milk by use of the gel filtration technique. These lipases have been partially characterized in an effort to establish if they are unique to the milk or if they perhaps originated from breakdown of mammary cells.

The research at the Institut National de la Recherche Agronomique, Paris, France, under a P.L.-480 grant on rennin activity and the interactions of milk proteins has produced explanations of some of the phenomena occurring in cheese-making practices. The strict specificity of the clotting activity was confirmed and led to explorations of the exact nature of the chemical bond sensitive to rennin. An important salt effect on rennin activity was revealed. When salt concentration was increased from 0.5% up to 2.5%, rennin activity was considerably reduced.

3. Nucleic acids. Physical-chemical studies on the structural organization of ribosomal RNA (H-RNA) are elucidating the structure of H-RNA from various sources, including rat liver and cow mammary gland. Future work will be carried out with H-RNA samples from cows, bacteria and plants.

Research in progress at the Centre de Recherches sur les Macromolécules, Strasbourg, France, under a P.L.-480 grant on the mechanism of the degradation of DNA by acid deoxyribonuclease has been advanced by the completion of work on the chemical composition of spleen acid deoxyribonuclease. It was demonstrated further that an enzyme from Escherichia coli, endonuclease I, attacks DNA in the same way as acid deoxyribonuclease.

This furnishes another valuable tool in elucidating the role of this enzyme in DNA metabolism and in probing the relationship between genetic factors and the synthesis of proteins.

4. Heat-resistant spores. The cell-free biosynthesis of dipicolinic acid (DPA) by extracts from sporulating cells has been confirmed and purification of the synthesizing enzymes is going forward. Hydrazine is lethal to bacterial spores and makes the spores stainable without release of DPA and hence provides one of the first opportunities to determine the localization of DPA in spores.

D. Milk Fat, Cheese and Nonfat Milk.

1. Milk fat. Results on encapsulation of milk fat as a means of attaining flavor stability have provided capsules containing anhydrous milk fat in edible membranes of gelatin-gum arabic, gelatin-casein, gelatin-sodium alginate, gelatin-carrageenan. Progress has also been made in separating and drying the individual fat-containing capsules.

Contract research on the effect of the diet of the cow on milk composition indicates that cows on lower levels of feed concentrates produce a more highly saturated fat.

2. Cheese. The flavor of low-fat cheese has been improved through the addition of small amounts of  $\gamma$ -lactones and by ripening the cheese milk with bacterial starter an hour or more before setting the curd. The laboratory procedure for making ripened low-fat cheese was scaled up and further tested in the pilot plant at Beltsville. The results of limited informal consumer tests with the cheese were favorable; several hundred persons now have sampled this cheese. Under contract research by the Ohio Agricultural Experiment Station it was found that the intensity of desired flavor in Cheddar cheese is related directly to the concentration of active sulfhydryl (SH) groups formed during ripening. The formation of active SH groups is related to the xanthine oxidase activity, which was greatly reduced by either pasteurization or hydrogen peroxide treatment of the milk. This discovery provides a chemical base on which to develop practical means of improving the kind, intensity and uniformity of cheese flavor. The adverse effect of heating milk or treating it with hydrogen peroxide emphasizes a need for a means of developing flavor in cheese made from pasteurized milk.

Scientists working under a P.L.-480 research grant at the National Dairy Research Institute, Karnal, India, have reported promising results toward production of bacterial enzymes as substitutes for calf rennet in cheesemaking where its use is restricted by religious beliefs in large areas of the world. Four hundred microbial rennets were prepared and studied for their possible use in cheesemaking. Five emerged as potentially good rennet producers. An additional highlight of this study was the discovery that the phytic acid contained in wheat bran, a cheap agricultural byproduct, greatly stimulates enzyme production. It is expected that, as a result of this and related research, bacterial rennets will eventually be produced industrially and that their use will lead to new domestic and export markets for cheese among groups with special food problems and prejudices.



Cheese technologists at the College of Agriculture, Olsztyn, Poland, working under a P.L.-480 grant, have acquired valuable information concerning the chemical changes occurring in Tilsit and Trappist cheeses during ripening. The decomposition of citric acid in Trappist cheese was proved most intensive during the first few days of ripening, continuing slowly thereon for about three weeks more. Electrophoretic and chromatographic studies on protein decomposition during the ripening of Tilsit cheese proved that the enzymatic action of rennet initiated in milk curd continues also in cheese. Protein degradation proceeds on a considerable scale both in the insoluble and soluble fractions of para-casein.

P.L.-480-supported research at the Kaira District Cooperative Milk Producers Union, Ltd. (Anand, India) is showing that the low fat content of native whole buffalo milk can be standardized with reconstituted skim milk for cheese-making purposes. The resulting cheese product has been improved to the point where body breakdown and analytical results are satisfactory, but flavor development is very slow even up to the age of six to nine months.

3. Nonfat dry milk. Equipment capable of concentrating skim or whole milk to as high as 60% solids for foam-spray drying demonstrated the advantages that may be realized by commercial plants upon adaptation of this new drying process, especially for nonfat milk. Foam-sprayed dried nonfat milk can be compressed into packages that would save about 33% in shipping space, without loss of its characteristic instant properties.

Results of contract research at the University of Wisconsin on the use of nonfat dry milk (NDM) in continuous bread-making indicate that NDM can support active fermentation by bread yeast. However, the extent of heat treatment during concentration of the milk solids is important. Yeast activity was increased greatly by NDM from concentrates that were heated at 185°F. for 30 minutes during concentration and further heated at 175°F. for 10 minutes after concentration.

4. Dairy processing equipment. Research has started on the use of liquified gas for foam-spray drying and on the development of a process and equipment for ultra-high concentration of milk. Studies are currently being made on the injection of liquid carbon dioxide and liquid nitrous oxide directly into the high pressure concentrate line feeding the nozzle of the spray drier. The use of the liquified gases would permit substantial economies, improve dispersibility and eliminate surface foaming during reconstitution of nonfat dried milk. The vacuum concentration of milk above 60% solids would provide further substantial economies in milk processing.

#### E. Radionuclides in Milk.

Under the research contract with Producers Creamery at Springfield, Missouri, it has been shown that 90-95% of the present environmental levels of strontium 90 can be removed from skim and whole milk without appreciably changing the flavor of the pasteurized product. The results indicate that the strontium-90 removal system is operable, efficient, and does not materially change the flavor of milk and that it is commercially feasible to materially reduce strontium 90 levels in milk.

#### F. Allergens of Agricultural Products.

After several years of intensive search for allergic serum, sera from five individuals allergic to cow's milk have been collected and are being studied to determine the protein fractions of cow's milk responsible for the milk sensitivity. No correlation has been found so far between the allergenic sensitizing capacity of various milk proteins and their previously determined antigenic capacity in experimental animals.

Good progress was made in the study of the antigenic specificity relationships of castor bean meal proteins, castor bean pollen proteins and castor bean allergenic fraction, CB-1A. Guinea pigs could not be sensitized to castor bean pollen antigens by sensitization with CB-1A. CB-1A had less than 0.025% crossed reactive potency with guinea pig sensitized with the pollen. Also, the cross reactive potency of castor bean pollen and castor bean meal antigens was less than 0.4% by the Schultz-Dale test and was 1.6% by a gel diffusion method of testing. CB-1A was separated into three main fractions and the three fractions characterized by disc electrophoresis. Also, CB-1A was fractionated by a semi-preparative method. Specificity relationships of the fractions were determined by gel diffusion analysis and the previous conclusion that chemically different fractions of CB-1A had an identical or common specificity was confirmed. However, a minor specificity was separated from the major specificity and some evidence was obtained for two additional specificities present in trace amounts.



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## III. MARKETING AND ECONOMIC RESEARCH

DAIRY PRODUCTS - MARKET QUALITY  
Market Quality Research Division, ARS

Problem. Modern marketing practices in the dairy industry have intensified the problems of detecting inferior lots of milk and of increasing the storage life of dairy products. Several kinds of insects and mites contaminate or damage dairy products during processing, storage, and distribution. To maintain quality of these products in marketing channels, research is needed on the factors influencing keeping quality; on developing new and improved objective quality tests for bulk milk and other products; on developing safe and effective procedures for preventing or controlling insect and mite infestations; and to find improved and simplified detection methods for antibiotic and pesticide residues in dairy products.

## USDA PROGRAM

There is a continuing program of basic and applied research aimed at developing new and improved methods for assessing the important quality factors in a variety of dairy products. At Beltsville, studies of the shelf-life of canned butteroil are continuing, work on chlorinated pesticide residues in dairy products is being expanded to cover other animal products and livestock feeds and forages; and new work has been started on estimation of moisture dispersion in butter and improving the direct microscopic count for bacteria in milk. Contract work under line project MQ 3-14(C) at the University of California at Davis on estimation of the protein content of milk by dye-binding has been completed. A contract under line project MQ 3-44(C) with the University of Minnesota on estimation of the quality of manufacturing milk has also been completed in the past year. Reports on both contract studies are being prepared for publication. The Federal scientific effort devoted to research in this program totals approximately 2.0 professional man-years.

There is a continuing program headquartered at Fresno, California, involving basic research in entomology and chemistry, directed toward the prevention of insect and mite infestation and contamination of dairy products in the marketing channels. The Federal effort on this program during the reporting period was 0.5 professional man-year. The entomologist on the program was at the University of Wisconsin during part of the period, doing graduate work under the Division's training program. Much of the cross-commodity research at Savannah, Georgia, reported in Area 13, "Insect Control in Marketing Channels," is also applicable to the problems in dairy products.



On June 1, 1965, a 2-year research contract in the amount of \$49,190 was awarded to the Stanford Research Institute for a study on natural attractants in certain dermestid beetles.

#### PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

The market quality research program at the State stations deals with the bacteriostatic action of fresh milk and its effects on cultured products; changes in milk quality during transport and storage; the effect of trace amounts of sanitizing agents on flavor and manufacture of dairy products; and developing techniques to predict shelf life of market milk. This includes rapid methods for enumerating psychrophilic micro-organisms.

The program concerns the development of rapid methods for measuring the gross composition of milk. Included in this area of research are efforts to develop methods which are rapid and accurate for protein, solids not fat, and for fat in dairy products such as homogenized chocolate milk.

Another phase of this program has to do with the effects of standardized milk on yield, grade, and quality of cheddar type cheeses; means of improving quality of cottage cheese, concentrated and dried products, butter and ice cream. Other research on quality concerns effects of processing on the flavor and quality of market milk.

One other phase deals with freezing point determination of milk under different management practices and different locations and also acid degree values as an index to rancid flavor in fluid milk.

A total of 10.5 professional man-years is devoted to market quality research on dairy products.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

##### A. Objective measurement and evaluation of quality

1. Protein Content. A manuscript for publication as a USDA Technical Bulletin was prepared by the contractor and is now being reviewed and edited.  
(MQ 3-14(C))

2. Manufacturing Milk. This project under contract with the University of Minnesota was completed. The final report provides, for the first time, comprehensive data on the quality of manufacturing grade milk and the

interrelationships among four grading methods (standard plate count (SPC), direct microscopic count (DMC), resazurin reduction test (RRT), and methylene blue reduction test (MBRT)) as affected by method of handling, geographical location, season, and other factors. The results showed that the RRT is probably the most suitable for a single grading test to be used to the exclusion of others, since it is inexpensive to run and seems to be less affected by geographical location, etc., than the MBRT or DMC. (MQ 3-44(C))

3. Stability of Anhydrous Butterfat. Samples put in storage in sealed cans at 32°, 70°, and 100° F. were opened after 1 year of storage. As compared to 6 months, the 32° and 70° samples showed more oxidation while little further change was noted in the 100° F. sample. A simpler and more reproducible method of carrying out the thiobarbituric acid test with butteroil was developed. All samples of butteroil appear to be still satisfactory for use after 1 year of storage although a slight oxidized flavor is evident in most. With ghee, a slight oxidized flavor was noted in the sample stored 1 year at 100° F. but samples stored at 70° and 32° F. were still good. (MQ 3-49)

4. Moisture Distribution in Butter. Conductance measurements with a commercial battery operated moisture meter showed that the instrument did not adequately differentiate among samples. A few samples with distinctly lower conductance appeared to keep a little better. (MQ 3-57)

5. Pesticide Residues. It was found that development under UV light of insecticide spots on thin-layer chromatograms could be greatly accelerated by steaming the plates prior to exposure to the light. This also improved sensitivity. Spraying with dilute hydrogen peroxide was helpful in preventing darkening from reaction of silver nitrate in the plate with fatty materials. Both florasil and carbon-celite cleanup procedures were usable with small samples of fat. As little as 0.01-0.02 ug of most common chlorinated insecticides in 0.4 g of fat could be detected on plates developed by the above technique, although because of slight interference from fatty materials, detection was more reliable at 0.05 ug or above. The cleanup and thin-layer chromatography proved satisfactory with poultry products also. (MQ 3-70)

## B. Prevention of insect infestation

1. Nonpesticidal Control Methods. The apparatus for aerating insects was redesigned to take up less space, hold a larger number of insects, permit continuous operation, and provide greater efficiency in collecting the natural attractant from a large number of dermestid beetles. The attractant is supplied to the Stanford Research Institute for their contract studies. A new type of highly efficient adsorption column was designed for collecting the attractant, and it may replace the conventional cold-trap method used up till now by other researchers. Progress has been made on developing a quantitative bioassay method to evaluate active fractions separated from the collected material and later on the evaluate the attractiveness of synthesized compounds. (MQ 1-32(C))



2. Improved Pesticidal Control Methods. By developing a new type of exposure cage and fumigation chamber arrangement it was possible to conduct controlled laboratory fumigation tests with methyl bromide against Acarus siro L., a common mite pest of cheese. The dosage in milligrams per liter required to kill 95 percent of the mites during different periods of exposure were: 16.8 for 4 hours, 8.1 for 8 hours, 5.2 for 16 hours, and 3.4 for 24 hours. The tests were conducted at 60° F. and 90 percent relative humidity. (MQ 1-6)

#### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

##### Objective Measurement and Evaluation of Quality

Moats, W. A. 1964. Staining of bacteria in milk for direct microscopic examination. A Review. J. Milk and Food Technol. 27 308-10. (MQ 3-44)

##### Prevention of Insect Infestation

Burkholder, W. E., and R. J. Dicke. 1964. Detection by ultraviolet light of stored-product insects infected with Mattesia dispora. Jour. Econ. Ent. 57(6): 818-819 (MQ 1-4)

MARKETING FACILITIES, EQUIPMENT, METHODS,  
CONSUMER PACKAGES AND SHIPPING CONTAINERS  
Transportation and Facilities Research Division, ARS

Problem. The equipment, work methods, and facilities used by many dairy plants are obsolete and the production per man-hour employed relatively low. A major factor contributing to this obsolescence is the development during the last few years of new types of equipment which can be brought under automatic control. Because of the investment required and the uncertainties of the returns they would obtain, plant operators have been reluctant to shift to automated equipment on a piecemeal basis. They also have been reluctant to build new plants because of a lack of guidelines and criteria on automated plants. However, studies indicate that it is possible in fully-automated plants to increase the productivity of labor 100 percent or more, to improve the qualities of the finished products, and to develop better management-employee relations. Engineering layouts and operating criteria therefore are needed for automated plants to provide guides to plant operators in making the shift from their present equipment and facilities. Most dairy plants lack the technological and engineering skills necessary to plan and develop suitable plant layouts and designs, or to select the types of equipment needed and the controls necessary for full automation. Automated equipment and processes for some types of dairy plants still largely are lacking or are nonexistent. Therefore, engineering research also is needed to develop equipment and processes for automating these plants in order to increase labor productivity and improve product quality.

It costs about 8 billion dollars a year to package food products, but without shipping containers and various other types of packages it would be impossible to move farm products efficiently from the widely dispersed areas of production through our complex marketing system to millions of consumers. New or improved packages and containers must be developed and evaluated to do this job more effectively. Continuing changes characterize the American marketing system.

USDA PROGRAM

The Department has a continuing long-term research program involving agricultural engineers and dairy technologists engaged in applied research to develop improved methods, equipment, operating criteria, plant layouts, and new and improved shipping containers for dairy plant operators.



Current research covers the development of layouts and operating criteria, based on current technology, for automated dairy product plants. It features the use of remotely controlled air operated valves, electronically-controlled devices, and highly mechanized equipment to facilitate automated processing and CIP cleaning in dairy plants. The work is carried out at Hyattsville, Md., and Columbia, Mo. Work in the Hyattsville, Md., office consists of checking, organizing, and preparing for publication a series of reports prepared under contract. Work at the Columbia, Mo., field location, which is conducted in cooperation with the Missouri Agricultural Experiment Station, consists of collecting, analyzing, and preparing for publication engineering data, from studies in both laboratories and commercial plants, needed for efficient utilization of automated procedures in fluid milk plants.

**The Federal effort devoted to research in this area is 2.6 man-years; 1.0 man-year for intramural work; 0.3 man-year for program leadership; and 1.3 for shipping containers for dairy and poultry products.**

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

##### A. Layouts and Operating Criteria for Automated Dairy Product Plants

At Hyattsville, Md., work was continued on the preparation for Department publication of a series of contract reports covering the development of layouts and operating criteria for different types of automated dairy product plants. Two of the six contract reports have been published. The status of the work on the four remaining reports in this series are as follows:

1. Plants Manufacturing Ice Cream and Ice Cream Novelties. At the end of the report year, the contractor's report had been checked, reorganized, and a revised draft for Department publication was completed. The significant results of this study were covered in a previous report.
2. Plants Manufacturing Cottage Cheese, Cream Cheese, and Cultured Milk and Cream. The manuscript for a report for Department publication based on the contractor's report was about 70 percent completed. Results of this study were covered in a previous report.
3. Plants Manufacturing Cheddar Cheese. Preparation of the contractor's report for a Department publication had not been started at the end of the report year. Results of this study were covered last year.
4. Plants Manufacturing Sweet Cream Butter and Dried Nonfat Milk. Work on this contract report has not progressed to the stage where significant findings can be summarized.

B. Increasing Efficiency Through Optimum Utilization of Automation in Fluid Milk Plants

During the last half of the year a research project designed to increase efficiency in fluid milk plants through optimum utilization of automation was initiated at Columbia, Mo. Specifications were written and orders were placed for automated control equipment which will be installed on the fluid milk processing equipment at the University of Missouri dairy plant. Both laboratory data from the University plant and data from commercial fluid milk plants will be obtained. At the end of the report year some information has been obtained on automated procedures in commercial fluid milk plants. However, the research has not progressed to the point where significant results are available.

C. Shipping containers and packages for dairy products

A preliminary review of four packaging systems (bottle, can, coated carton, and bag-in-box) revealed that a great variety of milk packages of many different sizes and shapes has been developed, often with little regard for dimension compatibility within or between systems. To show how compatible market units and dimensions can improve the marketing of milk, a series of six package models ranging from 1/2 pint to 2 gallons in size was constructed. A supply and demand model for milk packaging materials was fitted to price data for packaging materials at the dairy plant and to milk market information for milk consumed in the home. A preliminary report was prepared, describing the family of milk package models and their possible use in an automated transport system.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE PROGRAMS

None.